

Europe trickles into space

Next week's response from Europe to the US invitation to join in the space platform will be at best a compromise, at worst a nothing. Europe must make itself a policy.

PRESIDENT Ronald Reagan may have done Europe a service by his proposal that it should spend \$2,000 million on his \$8,000 million space station. At least he has knocked European heads together over an issue where European collaboration and expenditure certainly need to be enlarged if Europe is to play some part in the next stage of space technology, the building of large structures and the refurbishment of modular satellites in space. Sadly, the result, to be seen at a ministerial meeting of the European Space Agency in Rome next week, is likely to be a mess; but that is Europe's fault, not Reagan's.

The problem, essentially, is that the "European" space industry is not European, but national. National governments naturally back their national industries, with the result that decisions that should be commercial and market-led become highly politicized. This is mere pork-barrelling, and does not contribute much to the greater issues of European development.

European governments participating at Rome thus make great play with the issue of European "independence" from the United States in the control of, and access to, the space "infrastructure" represented by the proposed space station and its associated communications systems. On the one hand, this is presented as a great political issue, as by the French President M. François Mitterrand in a speech in the Netherlands last year. But on the other, M. Mitterrand is as much concerned about whether or not other states will back his French industry in the development of the \$900-million, winged "Hermès" mini-shuttle. Similarly, Britain will propose next week that technological (and possibly military) independence of the United States is only a distant issue, and that the question of putting Europe in space at all can be safely neglected for a time. Europe should back British Aerospace, the company which has proposed an experimental platform for polar or equatorial low-Earth orbit that would make no contact with the space station at all. Not being man-rated, the British flyer chalks up a much lower cost (perhaps by a factor of two) than the equivalent platform proposed by West Germany and Italy as part of the "Columbus" project. Britain, for the time being is therefore cool on men in space.

Independence

Columbus would come in two parts. There is a pressurized chamber, like Spacelab, for manned experiments, and a platform which can carry experiments as does the British Aerospace project, or dock with the pressure chamber to provide it with services. This would provide Europe with a poor man's independent space station — but only expensively. So one might expect West Germany, from self-interest, to take a middle position between France and the United Kingdom on "independence" from the United States. And this is exactly how the big countries of Europe line up, with Italy — which has a potential 25–30 per cent stake in Columbus — backing West Germany. Is this politics? Or is it business? And if the latter, is it good business? Either way, Europe seems unable to understand that the future prize may be much bigger than that now up for grabs.

That there is business to be won is attested by the Eurospace organization in Paris, which claims to speak for 60 of the largest aerospace companies in Europe. To 1993, Eurospace sees a market to European manufacturers of \$1,800 million a year in conventional launchers and satellites, and a microgravity market

of \$7,000 million a year thereafter. And though the second figure is based on some "highly questionable" US studies, there is at least a possibility that there will be a market big enough to require that competing investments should be assessed on more serious commercial grounds than seems likely at next week's ministerial meeting in Rome.

Even internal national squabbles will play their part there. In West Germany, for example, the finance ministry has taken months to agree to back both the development of a cryogenic motor for Europe's conventional Ariane launcher and Columbus, and has then taken its pound of flesh by cutting the budget of the research technology ministry (BMFT), which the cabinet in Bonn has now decided must find 30–50 per cent of the cost of the programme, putting space science at risk.

Veto

Britain's space scientists are also in trouble. Although there seems to be broad support for a 15 per cent contribution to Columbus (if the British Aerospace machine can be considered part of it), opinions are divided over whether to raise the ESA mandatory subscription, supporting basic science by, 50 per cent in real terms over the next decade or so, another point on the ESA agenda for Rome (see p.259). This internal British problem could well become a European problem, for unwilling payers have a veto. According to ESA, a 7 per cent annual increase in the subscriptions (now totalling around \$70 million, paying for one scientific launch every two years or so) would offer "reasonably relaxed" support for a detailed package of science missions for the next 20 years. This package, prepared over the past 18 months, has wide support in the scientific community in Europe as representing a minimum programme to keep Europe in business in space science. But according to ESA, the compromises it embodies would not survive a rate of increase of less than 5 per cent a year. Britain, however, offered only three per cent at the last ESA council meeting before Christmas. Will it be more generous next week?

Not only is three per cent insufficient to reach the Horizon 2000 threshold, but the British Science and Engineering Research Council, which has to pay the subscription, is in such straitened circumstances that it can hardly imagine paying even that. No wonder then that its council's head of science and (as it happens) the chairman of ESA, Dr Harry Atkinson, is talking hopefully of "leaky boxes". The leak he wants is out of the space budget of the Department of Trade and Industry, which will pay for participation in the space station, and into the budget of the council. Does he reckon without the stupefying bureaucratic isolations of departmental budgets in Britain, in which the tiniest leakage might seem a kind of public embezzlement?

Inflexibility probably also makes nonsense of the otherwise cogent arguments of Professor Martin Rees, the Cambridge astronomer, who says that space science is bound for decades to seem less cost-effective than the more modest schemes on which research councils think they should be spending money. Thus, Rees argues, the British contribution to the ESA mandatory budget should be hived off, to a British space agency, leaving the council room for better support for ground-based astronomy, among other things. All good sense, but no more likely to happen on that account. □



Where now with AIDS?

The time has come to stop being mesmerized by this new disease, and to do something about it.

IN society's lackadaisical defence (of itself) against the syndrome called AIDS, otherwise acquired immune deficiency syndrome, the article on page 277 of this issue is another landmark. Other equivalent landmarks will appear this week in other journals, *Cell* and *Science*. These three articles report the nucleotide sequences of particular isolates of the virus named HTLV III by Robert Gallo and his associates at the National Cancer Institute in the United States. *Nature* knows of a fourth viral sequence in the pipeline, just a few weeks behind, and there may well be others. But most readers of these articles will acknowledge that there is no obvious means by which any of them, or any combination of them taken together, will advance the treatment of a disease that, when full-blown, kills those affected with something like the certainty that summer is warmer than winter. There is a chance that the nucleotide sequences, or their comparison, will suggest some useful way of looking at the problem of treating this novel disease, but there is also a chance that they will do nothing of the kind. A bigger step was taken a few months ago, when it became clear beyond doubt that AIDS is caused, in part at least, by a virus that can be characterized in the laboratory and which can be recovered alive from body fluids as different as semen and saliva. Sequencing is merely better characterization, but in due course, as the permissible protein structures are defined, it will also no doubt have practical value for immunologists (people who, among other things, make vaccines).

The immediate interest of the three now-published nucleotide sequences will be in their comparison, and for a particular reason. Restriction enzyme mapping in several laboratories has already suggested apparently huge differences between the structure of one isolate of the virus and another. That discovery has seemed forbidding because it has suggested that different strains of the AIDS virus may differ from each other structurally, as do the viruses responsible for successive waves of influenza, a much more infectious entity. The defect in that conclusion is that restriction mapping, which consists of cutting pieces of DNA at specific sites, is ideally suited to telling small differences between largely similar genetic structures and that it may exaggerate differences between necessarily malleable genetic structures such as those of viruses. Nobody should be surprised if the differences between the genetic structures now published are of no biological significance, or are simple mistakes (what the electrical engineers would call noise). Equally, nobody should be surprised if the data suggest that the AIDS virus is so labile that the hope of making a universal vaccine is small.

So is it still an open question whether AIDS is a passing scare or a novel disease that has come to stay for good? In principle, yes; in practice, the weight of the evidence inclines to the opposing view that AIDS is a novel infectious disease that spreads only slowly, which for that reason has affected only small sub-communities but which, because transmission is mostly sexual, will in due course put all of us at risk. It is particularly disturbing that AIDS has now been reported among female prostitutes in New York. Retrospectively, it is now known that AIDS has been established among men and women for at least six years.

That then, is the bad news; there is no obvious barrier except a vaccine (whose construction would be attended by the immunological complexities already recognized as well as by the problem of making the first vaccine against a retrovirus) to the general spread of the disease. The good news is that the time-scale of the infectious process is long. Infection is clearly not overwhelmingly rapid. Not all those infected are certain to succumb. Certainly the analogue of the classical induction period is long, three or four years, perhaps. So there is a chance, even a good chance, that energy spent in the next few years on the understanding of the biology and the molecular biology of AIDS may literally help to avert the catastrophe as serious but more enduring than any of the horrid visions on which governments now

lavish dollars in units of tens of millions. True, the questions provoked by AIDS are more taxing than those provoked by, say, acid rain. But in an age when battling against imagined cataclysm has become heroic, is not the case for worrying constructively about AIDS the more urgent?

Other things not equal

Dollars go up and pounds go down. The underlying reasons are linked with productivity.

THE economists' standard way of disclaiming full responsibility for their conclusions, the English translation of the Latin *ceteris paribus*, has been widely used in the past few months, especially in connection with the United States budget deficit and its international ramifications. What the textbooks say is that a government that finances its public expenditure by borrowing what it cannot raise in taxes runs several risks. The most common is that the creation of domestic credit will stimulate inflation, a danger avoided in the United States largely because much of the borrowing has come from abroad. Another danger is that the need to borrow will keep interest rates high (because people must be tempted to part with their money). Another obvious risk for a government running a budget deficit (in excess of \$200,000 million this financial year) is that, other things being equal, the currency will depreciate as the supply of dollars overseas comes to exceed the willingness of people overseas to accumulate ever-larger piles of this commodity and its equivalents. But plainly, things are no longer equal. The currency that has fallen through the floor in the past few weeks is not the dollar but the pound sterling.

The economists' extenuating circumstances have dawned on people only slowly, but are in their own way also chilling. The willingness of people to lend money to a spendthrift government rests on the flexibility of the US economy in the past two years. The inflation rate has actually fallen (to 4 per cent a year) and industrial production has increased (by 6.4 per cent in a year, more quickly than in most industrial countries except Japan). Even so, the external trade deficit (\$122,000 million a year) is so high that it would keep most newly-inaugurated presidents awake at nights, while the money supply in the United States (up 10.3 per cent in a year) should have most people wondering how long overseas suppliers will be prepared to finance the unrequited import bill. The obvious danger is that the time will come when people's appetite for dollars is sated, when there will be a turnaround as uncomfortable for the United States as for others.

Britain, at the other end of the industrial spectrum, seems to have earned all the troubles that afflict the United States and those that still lie ahead of it. The British economy has proved to be less flexible than most, with the result that British exports to the United States have not risen as quickly as those of France and West Germany in response to the favourable exchange rate. Indeed, industrial production actually fell in Britain last year (partly because of a ten-month coal strike), earnings and as a consequence unemployment are both increasing, as is the money supply.

The morals of this tale of contrasts for the US government are clear — reduce the budget deficit, let interest rates decline still further and let the dollar decline modestly in value (as it would). So much is the price of avoiding instability on a grand scale. The recipe for the British government is harder to follow, if only because it has been tried in various forms for at least twenty years. The problem is to find some way of making the British economy more productive and more flexible. Successive governments of all stripes have aimed at these objectives by a variety of financial instruments, during the past decade by concentrating on inflation and the money supply. The present government has also tried, more openly than any since the Wilson Labour government of 1964, to encourage high technology. The snag, now as then, is that the weaknesses of the British economy lie not so much in these long-term goals as in the need somehow to keep body and soul together in the short run. Structurally, middle technology has been virtually destroyed by the events of recent decades. So long as that remains the case, people will go on selling sterling. □

Japanese budget

Good year ahead for science and technology

Tokyo

ANOTHER austere budget is on its way for Japan in 1985. Science and technology seem exempt from the general gloom, however, as the government struggles with its colossal deficit. Draft budgets for all the science-based ministries have been approved by the Cabinet, and show rises of between 3 and 12 per cent (see p. 256).

The new budget has yet to pass through the Diet, but cuts are not thought likely in the appropriations for science. The opposition parties will instead find plenty to disagree with in the planned reorganization of the health payment system and in the increases in the defence budget.

The increases for science, added to the ending of a few older big projects, are making it possible for all the science ministries to get several new schemes off the ground.

Science and Technology Agency (STA)

Several years of massive expenditure on the preparations for the Expo 85 International Science Exposition are now practically over. With the opening scheduled for 17 March, expenditure for the 1985 fiscal year will decrease by 65 per cent, providing some spare funds that can go elsewhere.

A big increase is made possible for Special Promotion Funds — essentially equivalent to the grants from the Ministry of Education, Science and Culture (MESC) in aid of research and used to support a host of small projects. Indeed, although the fund was set up only a few years ago, it is now worth nearly 20 per cent of MESC's grants.

The really big news for STA, however, is that a hefty budget allocation (1,400 million yen) has been made for the design of modules to comprise a part of the US space station. No final decision has yet been made on the extent of Japanese participation in the US project, but this first move makes likely a major effort later; Mitsubishi Heavy Industries is to be the main contractor.

Money has also been provided for the HII rocket (Y7,300 million and guarantees of further sums over the next few years for the rocket, and Y1,500 million for further research on its cryogenic engine). So despite criticism that the project is merely duplicating other nations' achievements and that satellites could be launched more cheaply by the United States or Europe, it seems that Japan will really insist on having an independent launch capability by the early 1990s. The rocket will be able to carry only a modest 2,000-kg satellite into orbit and it will not be able to handle the huge communications satellites now coming into use — but that still leaves a large part of

the satellite market (perhaps as much as 85 per cent) open to a potential Japanese competitor.

The new rocket does not exhaust STA's plans for massive new hardware. Money is also being provided to design a new deep-sea diving vessel. Four years ago, the "Shinkai 2000" submersible made a successful 2,000-metre dive; now work on a manned vessel capable of reaching 6,000 metres is to go ahead at the Japan Marine Science and Technology Centre.

In basic science, there will be a considerable increase (to Y2,500 million) in the Institute of Physical and Chemical Research (RIKEN)'s nuclear research budget. This will allow construction to continue of a heavy-ion cyclotron — planned to be the most powerful in the world.

Heavy ions also feature in another continuing STA programme for the medical use of accelerators. The project, at the National Institute of Radiological Science, is using heavy ions, including those of carbon, neon, silicon and argon, to treat tumours, apparently with some success. The only other such facility is at the Lawrence Berkeley Laboratory in the United States, although a laboratory at the University of Alberta, Canada, is expected to be set up soon.

In the biological sciences, Y187 million has been provided to set up a gene bank — all the rage this year, it seems. Both the Ministry of Agriculture, Forestry and Fisheries (budget Y391 million) and the Ministry of Health and Welfare (budget Y107 million) have also just set up their own gene banks.

Modest gains are also seen for nuclear energy research and project *Monju*, the prototype fast breeder reactor, gets a considerable budget increase — but not enough to stop the project falling well behind schedule.

Project ERATO — Japan's bid to stimulate creativity through the encouragement of fairly eccentric group research (see *Nature* 305, 373; 1983) — will take up two new themes this year: one on the physics of the nanometre region (in preparation for yet smaller devices) and one on atomic interactions at surfaces.

Ministry of Education, Culture and Science (MESC)

Grants in aid of research increase a little — but only a little more than the rate of inflation. Postdoctoral fellowships will become available for the first time. Only 72 (instead of the expected 200) of the Y200,000 (US\$800) a month fellowships will actually be offered (and another 72 of lesser worth for those still studying for a doctoral degree) but the holders will be en-

titled to apply for a considerable research grant, the first time that MESC has taken a serious step towards providing independent research support for young people. It is hoped that the number of fellowships will be expanded to 500 by 1989 but there are some doubts — even the funds for this project were at first refused by the Ministry of Finance.

Increases in funding have also been made for a number of categories of grant and numerous new projects launched. One project scheme — probably unique to Japan — is that of *tokutei* research. Grants are given to a subject area rather than to a specific group and anyone working in that area becomes entitled to apply for a part of the funds — the aim being to give a boost to a whole sub-discipline (sometimes involving more than 100 people) rather than to one research team. Among the nine new projects to begin this year are those on dynamic processes of material transport and transformation in the Earth's interior, the role of calcium ions in cellular regulation and nucleic acid conformation and its recognition. Total funds available run to Y5,300 million.

MITI

For MITI the big news is that a new "basic technology research promotion centre" is to be set up. The catch is that it is to be done in partnership with the Ministry of Posts and Telecommunications which had been lobbying for a similar institution to promote sophisticated regional telecommunications systems. The new centre will provide loans for basic research by private industry and set up new joint research projects. Relations between the two ministries are at an exceedingly low ebb as they struggle to gain control of computers and telecommunications research — the Ministry of Posts being badly wounded by the privatization of the telecommunications monopoly NTT — so it is expected to be some time before the organization is under way.

Two new-year "large-scale industrial projects" are also to be launched by MITI, one on flexible data base systems and one (the "aqua renaissance" project) to find ways of purifying and recycling water from industrial waste. Each will receive Y20 million in their start-up year. A separate project (budget Y70 million) for "next generation industries" will also begin for development of materials for optical components that will soon sit in optical fibre transmission lines and, one day, in the optical computer.

Alun Anderson

Correction

In the article on the Austrian dam on the Danube (*Nature* 17 January, p.171), it was incorrectly stated that the Danube Commission had set up an ecological study. That has in fact been done on the initiative of the Austrian and Czechoslovak governments. □

US telescopes

Uncertain future for 100-inch

Mount Wilson (California)

THE Carnegie Institution of Washington has shunned the recommendation of a scientific committee that it should continue to support the two solar towers and the 60-inch telescope at Mount Wilson observatory until an alternative sponsor is found. The institution announced last week that it will end its support for the 60-inch telescope and the solar towers by 1 July 1986, and confirmed previously announced plans to mothball the 100-inch telescope on 1 July this year unless another operator steps forward.

The scientific committee, chaired by R. Grant Athay of the High Altitude Observatory, records "profound dismay" at the "seemingly abrupt" decision to close the 100-inch telescope and the solar towers. It also describes as "impractical and unrealistic" the suggestion that the 60-inch telescope could be supported entirely by external grants, a conclusion endorsed by users of the instrument who sent evidence to the committee. The Carnegie Institution's announcement last week made no reference to Athay's report, which was undertaken at the request of Dr George Preston, director of the Mount Wilson and Las Campanas observatories.

Mount Wilson is not suited to deep-space

observations requiring dark sky conditions because of light pollution from nearby Los Angeles. But Athay notes that there are many "frontier problems" which are unaffected by light pollution and which could benefit from the excellent seeing and the long periods of uninterrupted clear weather at Mount Wilson. High-resolution spectroscopy is not limited by sky brightness and photometry and low-resolution spectroscopy can be done usefully by subtracting sky light. Important solar and stellar synoptic programmes now in progress would suffer from even a temporary interruption to observations. Furthermore, the Coudé spectrograph on the 100-inch telescope could be the best of its type in the world, and the observatory may be ideal for speckle interferometry and variable baseline stellar interferometry.

Athay's committee urges Carnegie to set up a blue-ribbon panel to oversee the transfer of the Mount Wilson observatory to another organization that can make good use of the equipment and the huge collection of observational records. But it is still not clear whether any of the several interested groups will be able to put forward a convincing plan for the observatory in time to prevent the shutdown of the 100-inch telescope in July. Carnegie has undertaken to keep the telescope maintained and exercised after July for an unspecified period.

One interested group, the Mount Wilson Corporation for Research in Stellar and Solar Physics, is trying to raise \$10 million, the earnings from which would support basic operations at the site. The founder of the corporation, George Roberts, expresses cautious optimism that the goal will be reached: negotiations are in progress with a major foundation. Roberts says a deal to provide the \$9 million still needed will be concluded "within 60 days or not at all". The Smithsonian Institution and the Jet Propulsion Laboratory have both previously expressed interest in a consortium to manage the observatory, and Roberts claims support from several prominent scientists and industrialists for his endeavour.

The Carnegie Institution intends that savings made by withdrawing from Mount Wilson — about \$750,000 per year — will be used to expand facilities at its Las Campanas observatory in Chile. Preston says the Carnegie Institution will increase its total spending on astronomy in coming years. New instruments are being fitted to the existing telescopes at Las Campanas — which has "unsurpassed" dark-sky conditions — and there have been initial discussions on a proposed new 8-metre single mirror telescope at the site. This instrument, like other planned telescopes, would use new technology being developed by Roger Angel at the University of Arizona.

Angel's mirrors are spin-cast in parabolic shape and consist of a lightweight glass honeycomb sandwich. Angel hopes that his recently-devised computer-actuated lap will solve the problem of polishing deep parabolic dishes to an accuracy of 1 micrometre and plans a mirror with an $f/1$ focal ratio. If it works — the technology is not yet proven on a large mirror — the cost of building telescopes with very large mirrors would fall dramatically. Preston will say of the proposed new telescopes only that "it's big", and he discourages speculation.

Tim Beardsley

Indian electronics

Transfer halted

New Delhi

INDIAN scientists have stalled a move by their government to acquire high polysilicon technology and equipment from the United States for the expanding indigenous electronic industry and photovoltaic programme to harness solar energy. A review of the matter has been ordered by the new Prime Minister, Rajiv Gandhi. It is a temporary setback to the agreed first transfer of US high technology to India.

Hemlock Semi-conductors Inc. of Michigan had signed an agreement with the Indian department of electronics last April to provide polysilicon technology for the proposed National Silicon Facility to be set up in Baroda in western India to produce high purity silicon crystals. The deal ran into rough weather at both ends. The US Department of Commerce declined to issue an export licence until a memorandum of understanding was signed between the two governments in November, after US suspicions about sensitive technology finding its way to a third country, implicitly in the Soviet bloc, had been allayed.

Indian scientists, on the other hand, say that the import of US technology is a disincentive for the indigenous research effort to develop the required technology. Already a two-tonne-a-year capacity polysilicon plant has been set up, based on process know-how from the National Chemical Laboratory at Pune. The process can be upgraded to produce silicon of high purity. Opponents also argue that the Indian silicon requirement will not exceed 20 tonnes a year in the next 10 years, so the proposed 200-tonne Baroda plant costing 90 million rupees will be unnecessary. Moreover, Hemlock's technology based on the Siemens process is said to be outdated and costly, so that it will not be possible to export the excess Baroda production at a competitive price.

The controversy has political implications for the national governments of both India and the United States. Mr Gandhi has therefore sought first a review by the political affairs committee of his ministry. Opposing scientists are keenly awaiting the eventual verdict of the full cabinet.

Sunil Suraf

Japanese science budget

	Yen (thousand million)	Percentage increase or decrease
Science and Technology Agency		
Total research-related budget	420.9	+ 3.1
EXPO '85	9.4	-65.0
Special promotion funds	7.3	+ 12.3
Space	91.5	+ 6.7
Nuclear energy ¹	254.3	+ 8.5
Cancer campaign	8.4	+ 10.4
Disaster prevention	2.1	-
ERATO	2.5	+ 11.9
Ministry of Education, Culture and Science		
Grants-in-aid of research	42.0	+ 3.7
Ministry of International Trade and Industry		
Total research related budget ²	193.2	+ 12.6
Basic technology promotion centre	10.0	-
Basic technologies for next generation industries	6.4	+ 11.0
Large-scale industrial projects	14.8	+ 13.3
Sunshine project	39.8	+ 8.1
Moonlight project	11.1	+ 15.6
Fifth generation computer project	4.8	- 5.8

1. Not including special account for nuclear plant siting
2. Regional science promotion budgets etc. are extra (~15%).

US creationism

Louisiana law thrown out

Washington

THE Louisiana law requiring "balanced treatment" of evolution and creationism in the public schools has been found unconstitutional by a federal judge. This is the second time since 1982 that such a state law has been thrown out by a federal court. And because this time the decision came in the form of a summary judgment, finding that the law is unconstitutional, other states are unlikely to test the courts further. No other state at present has a creationism law on the books.

The Louisiana attorney general has announced that he will appeal Judge Adrian Duplantier's decision to the federal circuit court, but meanwhile the state has agreed not to begin enforcing the statute.

The plaintiffs in the case, represented by the American Civil Liberties Union (ACLU), argued in their motion for summary judgment that no trial was necessary because no facts were in dispute. Judge Duplantier agreed. "Because it promotes the beliefs of some theistic sects to the detriment of others", he wrote in a ten-page decision, "the statute violates the fundamental First Amendment principle that a state must be neutral in its treatment of religions." Duplantier said that it was not necessary to repeat the lengthy 1982 trial of Arkansas's similar statute, in which dozens of expert witnesses testified as to what constituted science. "Whatever 'science' may be", Duplantier wrote, "'creation', as the term is used in the statute, involves religion, and the teaching of 'creation-science' and 'creationism', as contemplated by the statute, entails teaching tailored to the principles of a particular religious sect or group of sects."

The Louisiana law had avoided one pitfall of the earlier Arkansas statute by not providing specific definitions of "creation-science" and "evolution-science", thus making it a relatively easy matter to show that "creation-science" derives directly from *Genesis*. The Louisiana law simply declared "creation-science" to be the "scientific evidence for creation and inferences from those scientific evidences". Schools would not have been required to teach "the subject of origin", but if they had chosen to do so, equal time would have had to be given to evolution and creationism. A seven-member panel of "creation scientists" would have been appointed by the governor to advise local school districts on a suitable curriculum.

The "balanced treatment" approach was formulated by the creationist organizations (a model statute, drafted by the Institute for Creation Research, has been introduced in several state legislatures and was the basis for both the Arkansas and Louisiana laws) in an attempt to skirt earlier court decisions that laws banning the teaching of evolution are unconstitutional. Arkansas

had such a law on the books until 1969, when it was thrown out by the US Supreme Court. Opponents of the equal-time law argued that its obvious intent was to make the teaching of evolution so controversial and difficult that schools would choose to avoid the issue altogether.



Duplantier said it was unnecessary for him to consider the evidence that the state had presented, in over 1,000 pages of memoranda and affidavits, contending that creationism is science. A trial "could not affect the outcome". Duplantier said "we decline to put the people of Louisiana to the very considerable needless expense (in-

cluding fees of attorneys on both sides) or a protracted trial". The Arkansas trial cost the state not only its own legal expenses; it also had to pay ACLU's \$1.2 million bill for legal fees.

Martha Kegel, executive director of ACLU in Louisiana, said the decision in Louisiana sets a precedent for a "knock-out blow" to creationist statutes. The creationists, however, had already appeared to have begun shifting strategy after the Arkansas statute was voided in 1982 and after an earlier setback for the Louisiana law last year. (At that time, Duplantier held the law to violate the state constitution's delegation of authority over curriculum matters to the state's board of education but he was overruled on this point by the state Supreme Court; that was when the case came back to him to be heard on federal constitutional grounds of separation of church and state.)

Wayne Moyer of People for the American Way, a first-amendment lobbying group, notes that creationist efforts have been moving more and more to the local level. A number of local school boards have adopted creationist curricula; most of these have been successfully reversed by local action by concerned parents, teachers and clergy. Moyer also cited several recent cases in which individual teachers have been attacked by local creationists for teaching evolution. These cases, Moyer noted, are much harder to learn about and oppose.

Stephen Budiansky

Press goes off to Moscow

Washington

DR Frank Press, president of the US National Academy of Sciences, is in Moscow this week to discuss reestablishing a programme of scientific exchanges with the Soviet Academy of Sciences. A large part of the programme was suspended in 1980 by the National Academy in protest against the internal exile of Soviet dissident Andrei Sakharov.

Press was to have made the trip last summer, but cancelled it at the last minute amid growing concern that Sakharov was in failing health and might even have died. An earlier scheduled trip was cancelled following the Soviet downing of the Korean airliner in autumn of 1983.

Press said last week that he had informed the Soviet academy two or three months ago that he was now ready to rearrange the visit. He said that discussions with academy members and people who had recently visited the Soviet Union had convinced him that it was a good time to start talking again.

The programme suspended in 1980 provided for the academies to sponsor joint symposia that would bring together scientists from both countries. Such meetings had been going on since 1959. Since 1980, the only official contact between the two

academies has been through a Committee on International Security and Arms Control. During the period, however, the academy has continued to help to arrange individual scientific exchanges under a programme sponsored by the National Science Foundation.

These exchanges were cut almost in half in 1981, from 100 man-months per year each way to 56, but have remained steady since then. The reduction in 1981 reflected the Reagan administration's budget austerity measures rather than direct political sanctions, although the Reagan administration made no bones about its belief that the Soviets were getting more than they were giving in scientific exchanges between the two countries.

The academy is clearly sensitive about Press's visit, as little new information has emerged about Sakharov's condition since last summer. Press stressed that his discussions with the Soviet Academy will be only "explanatory". The academy would not confirm reports that Press would attempt to see Sakharov during his visit, although it is reasonably certain that Press will at least raise the subject of Sakharov's treatment when he meets A.P. Alexandrov, president of the Soviet Academy.

Stephen Budiansky

Anglo-Australian tests

British tell too little too late

BRITISH service chiefs flew in the face of safety warnings, and made servicemen "crawl, lie, walk and run" in radioactive dust from one of Britain's nuclear tests, the Australian Royal Commission was told in London last week.

Lord Penney, in charge of the test programme in Australia in the 1950s, told the commission that although he knew that the military had exposed men in such ways, he was not aware of documents warning the services of the dangers, in particular a document issued by the Admiralty in 1951 on gamma radiation which said that "all radiation dosage, however small, is harmful" and that men should be exposed to radiation only in the case of "demonstrable operational necessity". Lord Penney added that it would have been for the services to decide how to study the effects of nuclear explosions.

One military requirement of the British tests, according to Penney, was for a ground-burst weapon. Mr Greg James, representing Australian veterans, told the inquiry last week that the object had been to cause a "dirty" explosion resulting in large amounts of radioactive fallout, accomplished at a test called "Marcoo" at Maralinga in 1956. James said that to the British military, the test was successful in that it showed that dirty explosions would have had the potential to kill large numbers of people.

Referring to still-secret British documents that remain classified, Mr Peter McClellan, the commission counsel, described last week a row between scientists and the Ministry of Defence about the need for the second bomb in the MOSAIC test series, G2. Lord Penney said that he had believed it necessary because the first in the series had not produced the required yield. Penney said that the British government had promised Australia that neither test would exceed two and a half times the HURRICANE test (25 kilotons), and that the second would be fired only if the first was unsuccessful.

Mr McClellan said that the first test had indeed been a success but Penney was adamant that the second test had been necessary, despite the claim of officials who had described it as a waste of some £3-£4 million.

On testing underground, Lord Penney admitted to Mr Justice James McClelland, the commission's chairman, that his interest was stimulated only when a threat to atmospheric tests became apparent at the early test-ban talks in July 1958. But he also admitted that the minor VIXEN trials which began in 1959 could have been successfully contained in deep pits, thereby reducing the spread of radioactive material.

Dispute about fallout from the TOTEM 1 test (October 1953) continued last week. Mr McClellan asserted that Penney had

gone ahead with the test in atmospheric conditions so stable that the risk of fallout was increased, but Penney insisted that there had been no risk from the fallout of TOTEM 1.

The commission's search for British classified material also persists. Mr Robin Auld, the British government's counsel, said that his clients were now declassifying material and that they had produced more documents in the previous ten days than the Australian government had in six months. Mr Justice McClelland, however, tartly pointed out that it had been known for several months that the commission would be coming to Britain in early January, and that courtesy alone would have required "the mass of documents on the subject to have been collated, sifted and put into an easily accessible form". **Susan Watts**

Detour for Galileo

Washington

GALILEO, the US spacecraft due to begin its journey to Jupiter in May 1986, will make a detour around one of the larger asteroids, the National Aeronautics and Space Administration (NASA) has announced. The detour will add \$20-25 million to the cost of the mission which, NASA officials say, is a bargain price for what will be the first-ever close observation of a large asteroid.

A final decision will not be made until after launch; but if the detour is approved, Galileo will pass within 10,000 km of the asteroid, 29 Amphitrite. The plans involve mapping the surface with the spacecraft imaging equipment and probing the asteroid's mineral composition with spectrometers.

The Solar System Exploration Committee, a NASA advisory committee made up of space scientists, has for a long time been pressing for spacecraft exploration of asteroids as one part of an overall planetary exploration programme. From the Galileo fly-by, scientists hope to determine Amphitrite's mass, density, rotation rate, pole orientation and surface morphology. The data may also prove valuable in providing a basis for interpretation of ground-based observations of other asteroids.

Because of the extra expenditure of fuel necessitated by the detour, Galileo's arrival at Jupiter will be delayed three months; present plans call for the spacecraft to enter orbit around the planet on 10 December 1988. The number of orbits around Jupiter will also have to be cut, from 11 to 10.

NASA officials say the risks of bringing the spacecraft near the asteroid will be no greater than the risks that would be encountered in any event from passing through the asteroid belt on the way to Jupiter.

Stephen Budiansky

European anti-trust law

Ripping off red tape

Brussels

EUROPEAN companies (both indigenous and US or Japanese subsidiaries) jointly developing new products or processes will no longer need to spend time and risk untimely publicity by notifying the European Commission of each new joint venture. European Community anti-trust regulations obliging companies to notify the Commission of joint research and development agreements have been simplified. Fifteen years after the idea emerged, in the 1968 notice on cooperation between enterprises, there is now to be block-exemption from competition rules for agreements on joint research and development as well as the exploitation of results. Moreover, the regulation applies not only to research but also to joint marketing and production of the results of the research, an aspect championed by former research commissioner Etienne Davignon.

The new measure should be an important encouragement for European collaborative industrial research and development. There have even been some fears that the Commission's own Esprit programme on information technology would have fallen foul of the previous regulations. The block-exemption gives the official Community stamp to a practice common among the larger European companies undertaking research and development, and will in particular benefit small and medium-sized companies seeking joint research and production.

To avoid monopoly and to encourage diversity, however, the European Commission stipulates that the combined market share of production by cooperating companies should not exceed 20 per cent. But if companies do not sell products in competition with each other, there will be no restrictions on their collaboration in research programmes.

In addition, European companies marketing the fruits of joint research projects will also benefit by being freed from the requirement of active competition for the first five years, and for even longer while their joint market slice exceeds 20 per cent.

In order to qualify for the block exemption from anti-trust regulations, European collaborations must be embodied in a written research programme. Companies must also agree not to pursue individual research and development in the areas of joint research, that access to results and patent rights should be available to all partners, that agreements with third parties on, for example, licensing should not be undertaken without the other partners' consent, and that each company should have exclusive distribution rights in its own country.

Anna Lubinska

European Space Agency

Plans for the next decade

MINISTERS from eleven European nations meet in Rome next week to decide the future of the European Space Agency (ESA) for upwards of a decade.

ESA has placed a seven-point programme on the table which would raise its present \$650-million annual budget by 60–70 per cent. Yet only one of the points on the Rome agenda directly concerns the highly-

publicized invitation by the US National Aeronautics and Space Administration for Europe to participate in the US space station, due for launch in the mid-1990s.

The items ESA will put before ministers on 30–31 January are:

- A five-per-cent annual real increase in the mandatory science budget of ESA, to fund "Horizon 2000", a well-thought-out package of proposals designed to balance and satisfy the interests of Europe's space scientists for the next 20 years. Target budget: \$130 million a year, double present spending.

- A ten-year, \$1,300-million programme of Earth observation, with the launch of Earth Resources Satellite 2 for oceanography and meteorology in 1992–93, an advanced land observation satellite in 1994–95, and a second-generation meteorological satellite including an all-weather microwave system.

- A telecommunications programme, of which the major element, a \$850-million orbital data relay system (comparable with the US Deep Space Network), should be agreed before the construction phase of the space station begins, according to ESA.

- A \$55-million "technology" pro-

gramme.

- A microgravity research programme costing some \$55 million a year from 1988–92, to cover the use of the space station, a budget item that was ignored in ESA's "Spacelab" programme on the Shuttle.

- A space transportation system: in other words, the extension of Europe's launcher, Ariane, with a new cryogenic engine, HM60. This would cost \$1,750 million to develop over the period 1986–95, plus \$20–30 million a year for the "related elements".

- Finally, Europe's direct contribution to the US space station, which would consist principally of a manned laboratory called "Columbus", developed from Spacelab, and a linked man-rated experimental platform.

These are proposed by Germany and Italy at a price of \$1,750 million including development and use to 1995.

Britain has proposed a separate, automatic platform which its developers, British Aerospace, claim would be substantially cheaper than the German-Italian platform proposal.

And France has proposed that ESA develop Hermès, a man-rated mini-shuttle to be launched by Ariane at a development cost of some \$900 million to 1995. A proposal by British Aerospace to develop HOTOL, an air-breathing launcher that the British company claims would be cheaper to use than the "60s technology" Ariane, and cost \$550 million to develop, came too late to go on the ESA agenda for next week's meeting.

Robert Walgate

UK research councils

Geology to break away

ALTHOUGH the details of the UK Natural Environment Research Council (NERC) corporate plan have yet to be agreed, the staff at one of the council's largest institutes, the British Geological Survey (BGS), are already plotting in defence. The existence of this plan became known only last month, but it is likely to be approved at the next meeting of the council. One of its provisions is that research direction would be centralized at NERC's headquarters in Swindon.

A full meeting of senior officers of BGS last Friday determined, first, that BGS is opposed to the corporate plan and, second, that independence from NERC is its own preferred solution. A letter has been sent to all council members and assessors protesting about certain aspects of the proposed changes, but has been voluntarily embargoed until after the research council's meeting later this week.

In the slightly longer term, BGS is setting up a working party to establish a strategic

plan for its own future function. In essence, this could be an alternative to the NERC proposals. Of particular concern is the relationship between the three often conflicting functions of the survey: as a strategic arm of government, as a centre for applied earth sciences and in pure research.

BGS intends to present detailed plans, covering various financial options, to NERC before 1 April, the date for the first implementation of the proposed changes in management structure within the research institutes. As the largest of them, BGS believes that its working party could mould the shape of other NERC component bodies and guide the future course of their relationships with universities, government departments and industry. Long-serving senior officers of the survey describe the strategic plan as the single most important thing that the Geological Survey has decided upon during their careers. Just how NERC will react remains to be seen.

Peter Gambles

FDA

Ban on quack remedies

Washington

THE Food and Drug Administration (FDA) moved last week to ban the sale of two popular categories of quack medicines — hair growers and aphrodisiacs. In notices published in the *Federal Register*, the agency said that the products being sold over the counter for these purposes were medically worthless, though not necessarily harmful.

Most of the products in question have been on the market for years. Although new chemical substances sold as drugs must go through a lengthy premarket review by FDA to establish their safety and effectiveness as evidenced by laboratory tests and clinical trials, "grandfather clauses" in the food and drug acts and amendments have allowed over-the-counter medicines to stay on the shelves until FDA can prove them dangerous or worthless. The burden of proof in these cases thus shifts to FDA, making for a slow process.

The hair growers and aphrodisiacs were picked out, FDA officials say, because both have been heavily advertised and appeared to be areas of "considerable consumer fraud". A survey of magazine and newspaper advertisements of questionable health products found hair growers second only in frequency to diet nostrums.

Stephen Budiansky

Centrifugal China

DECENTRALIZATION in China is spreading from the management of the economy to that of science. Institutes of the Chinese Academy of Sciences must now be given greater decision-making power as the "highest reform priority".

Addressing a "working conference" of the academy on 13 January, Politburo member Fang Yi stressed that the academy's programme of changes announced in January must bring it to the "forefront of reform" of the country's science and technology structure.

Greater involvement with industry is the main aim of the reform. The proposed changes will allow qualified staff members of academy institutions to work in industry or higher education "so as to use their talent to the full". New technology development centres and companies are to be established to sell research results to clients in the production sector. Institutes concerned with applied science and technology will be financed by a bidding or contract system, while a special fund will be established for institutes dealing with basic research. A special fund of 10 million yuan annually will be established for awards to individual scientists.

Vera Rich

North Sea oil

Women not at sea

THE British oil industry is divided over the findings of a report published last November by the Equal Opportunities Commission, *Women in the North Sea Oil Industry*, which claims that the recruitment of skilled personnel for offshore geological work is regulated by managerial chauvinism, and discriminates against women.

The report is the result of a year's research by Robert Moore and Peter Whybrow of the University of Aberdeen. Although no official figures are available, it estimates that women employed in the British sector of the North Sea make up only 0.01 per cent of the 22,000 strong workforce, but it has to be said that this statistic is culled from head-counts taken at Aberdeen heliport. Five years ago, a Petroleum Training Board report found that women failed to progress far within the industry, were often over-qualified for the jobs they were doing, and expressed high levels of dissatisfaction.

Are attitudes changing? Not swiftly enough, according to the authors of the report. The volume of prejudice and misinformation circulated by the oil companies, they say, and regurgitated by careers officers in schools and universities, is bewildering. Women intent on careers as geologists and geophysicists, or even in catering, are discouraged and obstructed.

The oil companies, however, say that it is their policy not to discriminate. Shell's new platforms, now under construction and to be completed within the next three years, are equipped with smaller 2-berth cabins designed to accommodate women as 10 per cent of the workforce. Nevertheless, the report claims that many companies, anxious to disguise a restrictive recruitment policy, in the course of the annual "milk round" of aspiring university students, frequently interview female geologists even though they have no intention of taking them on. Interviewing procedures differ between the sexes. While men are examined on grounds of technical ability, women are interrogated about boyfriends and babies. Two contradictory images are assiduously fostered by the oil companies, it is alleged — that off-shore oil-rigs are a rough-tough man's world in which women have no place and that they are enlightened employers with whom women can be successful. Shore-based laboratory jobs offer slim prospects of advancement.

When unveiling the report, Patrick Walker of the Equal Opportunities Commission explained that it was to provide the basis for a general discussion that might precipitate government action to outlaw discrimination. One hope is that the government will apply Section 10(5) of the Sex Discrimination Act to the United Kingdom Continental Shelf. Professor Moore, co-author of the report, says that

successive governments have been eager not to do anything that would affect short-term production of oil. For the same reason, race relations laws do not cover the North Sea.

The oil companies, however, dismiss the report. Barry Freeman, personnel director at Chevron and chairman of the Employment Practices Committee of the United Kingdom Off-shore Operators Association (UKOOA), says it is "generally regarded as a fairly polemical document, and we don't like its presentation or its assumptions". A spokesman for Shell says that it was not "terribly well researched" and complains that his company was not consulted. (Professor Moore disputes this.) Countering claims that women are not allocated offshore duties on the grounds of gender, the head of personnel at Esso, whose partnership with Shell constitutes the largest operation in the North Sea, explained that technicians are required to serve a five-year apprenticeship before they are eligible. "It's a process of natural selection."

According to Professor Moore, however, it is not the oil companies which are the main offenders, but the contracting companies which recruit 75 per cent of those working in the oilfield. The companies explained that these comprise divers, drillers, helicopter pilots and other specialist service trades which normally exclude women. Yet diving companies do employ women, but only as instructors.

Theories of natural selection cease to

mean much when the British oil industry is compared with its Norwegian counterpart. Norwegian women are employed in a wider range of managerial and technical jobs than their British counterparts. The report concludes that "the Norwegian experience shows that women may be employed off-shore". Why is the situation in Norway different? Professor Moore pinpoints the Norwegian trade unions' partnership in North Sea exploration. "British recalcitrance over health and safety was in part a reluctance to recognize trade unionism in the North Sea." A thriving merchant marine tradition in Norway which involves women has meant a proliferation of highly skilled female mechanics, technicians, radio operators and so on.

Positive responses to the report's publication prompted last month's formation of UK Women in Geoscience. This informal group of women employed in the oil industry is coordinated from the University of Aberdeen. It was thought by its organizers that the oil companies were themselves thinking of setting up a committee to investigate and ultimately whitewash the sex equality issue. UK Women in Geoscience is intended to preempt this by broadening the debate. In Britain, as things are, the demand from women for North Sea jobs is small. Shell says that its recruitment of "girls" for its newly-created technicians' training scheme is directly proportional to the ratio of applications received, approximately 5 per cent. If that figure is undesirably low, *Women in the North Sea Industry* concludes, "parents, schools, universities and colleges bear a responsibility."

Hugh Barnes

Embryo research

Politicians prepare action

A BILL that seeks to outlaw experiments on human embryos and, possibly, the practice of surrogacy (see *Nature* 10 January, p.83) will receive its second reading in the British House of Commons on 15 February. A meeting last week gave Members of Parliament who support Mr Enoch Powell's private member's bill and who are worried by some implications of the Warnock Committee's report (see *Nature* 312, 389; 1984) a chance to put their case to interested members of the Commons and Lords with the aid of two visiting speakers. Dr John McLean, lecturer in anatomy and embryology at the University of Manchester, totally rejected the Warnock recommendations on embryo research, saying that the report reduces "the status of the human embryo to that of an experimental rodent, contravenes the code of medical ethics and must be rejected".

Robyn Rowland, a lecturer in social psychology who has recently resigned from Australia's *in vitro* fertilization programme, warned of the vulnerability of

infertile women to manipulative scientists who "promise they can create a child for them" and of the unconsidered effects of failure to fulfil the promise ("86 per cent of cases"). She also warned against developments such as the artificial womb and placenta, genetic manipulation and sex pre-selection.

The meeting unanimously concluded that non-medical scientists (that is, those not under the control of the General Medical Council) have too much control over legislation of the whole range of reproductive technologies, rather than medical practitioners and/or lay representatives of those directly affected. William Cash, the MP sponsoring the meeting, will seek to have a special select committee set up after Mr Powell's bill is read: researchers in the field (such as Dr R.G. Edwards) would be invited to give evidence to such a committee before legislation is introduced. Dr Edwards, regretting that he had not been invited to Mr Cash's meeting, nonetheless expressed willingness to put his case yet again.

Maxine Clarke

US biotechnology

Another joint venture

Los Angeles

YET another US corporation has joined the biotechnology bandwagon by pledging millions of dollars to help support a basic research laboratory. In exchange, the company will be able to work closely with leading molecular biologists and to profit from products or ideas stemming from the collaboration.

The arrangement is similar to many others that have been struck between academic research institutions and profit-minded corporations over the past seven or eight years. The trend has some scientists worried about academic integrity while others say the relationship is inevitable and healthy.

The newest deal is between Pittsburgh Plate Glass (PPG), a chemicals, coatings, glass and resins company in Pennsylvania, and the Scripps Research Clinics of La Jolla. PPG announced on 18 January that it would contribute \$120 million over the next 15 years to a biochemical programme. Depending on how the programme develops, Scripps could receive most of the money.

At a press conference in La Jolla, PPG president Edward J. Slack explained his company's goals. "We've been in the agricultural chemicals business since the early 1940s, and we wanted to expand that effort", he said. So the company searched for a first-rate molecular biology laboratory in which to relocate its own scientific team. Scripps was chosen.

PPG scientists will work side by side with Scripps scientists, renowned for protein analysis through peptide synthesis. Scripps researchers will isolate proteins specific to certain weeds. PPG scientists will then target herbicides to inactivate the selected proteins. "We believe we can develop highly effective agricultural chemicals without side effects that may damage the environment", Slack says.

Hitherto, Scripps scientists have generally not worked on plant materials. A new plant molecular biology laboratory will be built to house the new programme.

Scripps was given its present molecular biology complex thanks to a similar deal signed nearly three years ago with Johnson and Johnson (J&J). Pharmaceuticals house scientists work closely with Scripps scientists in biomedical research. Although J&J has never said how much it contributes to Scripps each year, a major portion of the \$6.2 million that Scripps received last year from industrial sponsors is said to have come from J&J.

In their agreement, J&J scientists get first review of scientific papers flowing from the molecular biology laboratory. They can select items to be patented, although Scripps owns the patents. J&J has exclusive licensing rights. No patents have yet been announced by either party.

Most major research institutions seven or eight years ago realized that, as National Institutes of Health funding began to level off, outside funding was needed, says Scripps director Charles Edwards. "It was obvious to turn to industry. It is difficult [for industry] to put together the kind of research establishment we have here. So the marriage of the two is natural".

Critics of such marriages, however, raise several points. The nature of biology is changed. Every academic biologist of quality is signed up by one company or another. The fear is that the direction of molecular biology will be diverted from following the goals of individual scientists to following industry's goals.

The tradition of biology teaching is changed. Top scientists tend to support top students. The message to today's young biologists is that in order to be smart, you have to be rich.

Trust in universities could be eroded. Public funding of academic institutions has meant that citizens could turn to universities for unbiased, disinterested advice. When the advice is no longer disinterested, the public is cheated.

Companies could invoke the right of keeping information a trade secret (rather than taking out a patent) which would deter the flow of information through the academic community.

Finally, academic researchers cry "foul" when the Department of Defense asks for prior review of any research but allows private enterprise such prior review with no complaint.

Sandra Blakeslee

Soviet refusniks

Traps of black market

Paris

JEWISH *refusnik* scientists in the Soviet Union are more than ever dependent on colleagues abroad for moral and professional support, according to an international round table held here last week. But some forms of help should be avoided. One such suggestion, made at the press conference following the round table, brought an impassioned warning from Dr Aleksandr Voronel, himself an ex-*refusnik* and the founder of the Moscow "Sunday seminars", that some actions could make the *refusniks'* already unpleasant plight

much worse, even exposing them to risk of long-term gaol sentences.

The suggestion in dispute was that visitors to the Soviet Union should take with them technically sophisticated goods which the *refusniks* would then sell privately. Since many *refusniks* have been barred from professional work since filing applications to emigrate, some more than ten years ago, and have long-since exhausted their savings, the notion that they might be helped by selling western goods privately obviously seemed a good idea to one naive well-wisher. But although such "left-handed" trading is a feature of Soviet life, what might be tolerated in a citizen otherwise in good standing with the authorities might be unacceptable in a *refusnik*.

Such naivety, in a scientist sufficiently interested in the fate of the *refusniks* to turn up at a meeting on the subject, bodes ill for the knowledge of the less committed. Indeed, even the organizers of the round table, the International Committee of Scientists for Soviet Refusniks, seemed at times a little hazy, departing from the *refusnik* consensus that they must avoid being confused with Soviet dissent to the extent of arranging for the one session about Dr Andrei Sakharov who, although he has a Jewish wife, is not a *refusnik*.

According to reports presented last week, Jewish emigration from the Soviet Union is now virtually at a standstill, and there has been a considerable increase in the number of trials of *refusniks*.

What Western scientific colleagues can do to help is not entirely clear. Last week's round table rehearsed the old debate between boycotting of scientific contacts with the Soviet Union and personal pressure on Soviet colleagues within the framework of normal scientific discourse. The International Committee last week adopted a list of long-term cases for whom special efforts will be made.

Vera Rich

How to help

THE *refusniks'* own views on Western help as expressed in a letter to the round table participants are as follows.

● **Their only aim is to go to Israel. If direct travel there were a condition of emigration they would be delighted to accept it. (In other words, they do not want to "drop out" in Vienna and go to the United States, a growing practice in the later 1970s, and one which the Soviet authorities sometimes cite as a reason for refusing visas.)**

● **Western colleagues should press for them to obtain visas to emigrate. Substitute concessions, such as reinstatement in their professional jobs, freedom of contact with colleagues and correspondence abroad and so on are unacceptable, and in any case are liable to be "ambiguous and temporary".**

● **At the same time, they "cordially invite" more colleagues to visit them and their seminar. They deplore the recent tendency among some Soviet scientists (not officials) who put pressure on Western visitors not to visit the *refusniks*, on the ground that this could be harmful to the visitors themselves. Such pressures, the *refusniks* say, are "devoid of all substance" and should be ignored.**

Vera Rich

Agriculture R & D

SIR — Without entering into the substance of the debate that W.S. Wise raised in his letter (*Nature* 3 January p.8), there is one comment I would like to make.

There are, of course, many ways of combining measures of volume of research and development with other statistics, such as Gross Domestic Product or the number in the population. Some may illuminate issues such as how the United Kingdom compares with other similar nations in the field of research and development funding. One such combined indicator was quoted in the *Annual Review of Government-funded R&D*. It concerned the volume of expenditure by government on research and development specifically to improve productivity and competitiveness of an industrial sector by comparison with the importance of that sector to the nation's economy. The *Annual Review* gave figures for a number of countries showing how many units of national currency the government spends on research and development for an industrial sector for every thousand units of currency earned by that sector for the economy. The purpose was to compare UK government priorities for industrially related research and development with those of other countries, taking account of the different economic significance of each industrial sector to each country.

To have added in university funding for agriculture research and development would simply have resulted in the comparison of some other quantity: university priorities in basic research are not necessarily the same as government industrial sponsorship priorities. Similarly, to have used population as a normalizing factor results in a less relevant comparison. I should also add that the Organization for Economic Cooperation

and Development (OECD) was the primary source of data in order that the United States and Japan could be included in the comparisons; as Mr Wise acknowledged, the OECD data do not allow the comparison that he has carried out for European countries.

I would not argue that either of the measures Mr Wise proposes are non-valid or necessarily less valid than the ones chosen for the *Annual Review*. I would simply suggest that they are different, and do not quite address the issue that the review was considering.

I would also like to point out that the published *Annual Review* represents the government's desire to make available generally the factual material that was used in the 1984 review, and so to facilitate informed debate such as the one Mr Wise has initiated. It would be inappropriate for such a document, which is essentially statistical in nature, to have attempted to capture a "spirit of enterprise" as he suggests at the end of his letter.

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Conditions for disarmament

SIR — R.I.P. Bulkeley's objection to our defence-protected build-down (DPB, *Nature* 312, 301; 1984) is a valid one if, as he assumes, the defence is deployed to protect cities. However, in our longer descriptions of our proposal (for example, *The Bulletin of the Atomic Scientists* 40, 18-23; 1984) we make clear that DPB would initially be deployed only to protect the strategic, land-based force which is perceived by the other side as posing a first-strike threat. This defence might be short-range terminal anti-ballistic missile (ABM) interceptors, swarm-jet or even decoy holes (which, though passive, achieve the same objective as do active terminal defences).

Thus even if the side which has adopted DPB strikes first with only 90 per cent of the force it had before deploying its terminal defence, the "ragged" counter-attack would still be sufficient to destroy the attacker's cities. In short, we do not visualize DPB as eliminating deterrence by Mutually Assured Destruction (MAD), but simply as starting a process which, at each stage, lowers the level of violence at which MAD operates.

We do not insist on precise mathematical equivalence at each stage. DPB, confined to terminal defence of land-based missiles, makes unilateral reduction of the first-strike weapons politically feasible. We should expect that such a move (confined to protection of missile sites) could not be

perceived as threatening by the other side since, if first strike were intended, it is illogical to dismantle some of one's first-strike weapons. Whether de-escalation under DPB continues depends, of course, on each side's recognizing that reduction of first-strike weapons is the best possible evidence that a first-strike is not intended. This, coupled with constraint on deployment of ABM around cities, could lead to a gradual de-escalation of both sides' offensive nuclear arsenals. That stability still depends on MAD is a dismal reality; perhaps, if DPB leads to sufficiently deep reductions in first-strike weapons, MAD might eventually be replaced by Mutually Assured Survival—but this is far from today's reality.

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Human embryos

SIR — So the House of Commons opening debate on the Warnock Report was "muddled", "down-at-heel" and peppered with some laughable "misunderstandings" (*Nature* 29 November, p. 389). Of course those who were hoping — as you were — that liberal opinions on human embryo experimentation would win the day, will have been disappointed by the mainly pro-life speeches. The fact is that there are many others (recent polls and petitions would suggest a majority of millions in the United Kingdom) who are saying "No" to most of Warnock's recommendations and the research-must-go-on ethic. As you know, most are persuaded, from scientific as well as other evidence, that human life begins at conception. The human zygote is therefore a fellow human being and, as a consequence, worthy of all the protection we afford ourselves. As a leading article in *New Scientist* put it, "the idea of experimenting on human embryos leaves a nasty taste in the mouth". And as you rightly say (*Nature* 308, 1; 1984), "the sources of anxiety should be identified and recognized . . . but each of them must be met". If there really are convincing legal, logical, moral or scientific arguments that would make research on human embryos acceptable, then let's have them; clearly Warnock was not able to raise many.

To you, the opportunities in this field of experimentation may be "exciting and important"; to the great British public and many of your readers, that is not the measure of what is right and proper. These experiments are considered to be sinister and inappropriate and to be forbidden.

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Misquoted

SIR — In your report (1 November 1984, p.7) on my talk at the dedication of the Monsanto Company's new Life Sciences Center, I'm said to have "attacked the government practice of supporting work by a single investigator in a single department. . .". What I actually said, in the context of discussing interdisciplinary research, is "that the project grant mechanism implicitly assumes a single principal investigator working within a single department. That is increasingly not the case."

For the record, I continue to regard grants to individual investigators as the single most powerful reason for the sustained excellence of American basic research.

FRANK PRESS

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Towards fivefold symmetry?

Crystallography is in for a minor upheaval, with the recognition of forbidden icosahedral symmetry by both construction and experiment.

THAT crystals never have fivefold axes of symmetry has been part of the doctrine of crystallography since 1895, when Fedorov first classified the admissible symmetry groups. The reasons are straightforward. Consecutive rotations of an infinite crystal lattice in either sense about one or more symmetry axes must yield an identical structure, identically placed in space. But rotations by $2\pi/5$ in opposite directions about neighbouring fivefold axes would break this rule.

Grandly, it might be said that the inadmissibility of fivefold crystal symmetry says something about the character of two and three-dimensional space. Similarly, it is not possible to tile a plane with equilateral polygons or to fill three-dimensional space with icosahedrons, the regular twenty-faced polyhedra most easily described as a pair of opposed pentagonal pyramids fashioned from equilateral triangles and joined together, base to base, by a band of ten opposed identical triangular faces.

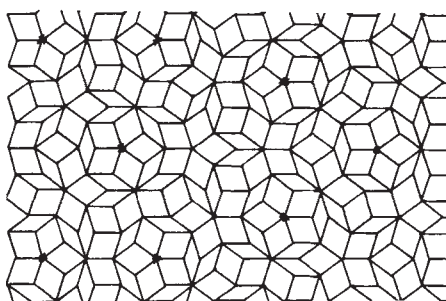
Both by accident and design, people have been whittling away at these restrictions for the past decade, ever since the demonstration by R. Penrose (Oxford) that, although it is not possible to tile the two-dimensional plane with regular pentagons, that can be done with a pair of specific rhombi so as to give a pattern with local fivefold symmetry (see figure). The price for this is that the pattern lacks the translational symmetry of a familiar lattice. The argument has been extended to three dimensions by A.L. Mackay (Birkbeck, London), who has shown that space can be packed by acute and obtuse rhombohedra (see, for example, *Physica* **114A**, 609; 1982). Again, translational symmetry is forfeit to the gain of local fivefold rotational symmetry.

The bearing of all this on real crystallography is far from remote. The textbooks are mostly about infinite and perfect crystal lattices, but real crystals may be small and imperfect. Indeed, X-ray diffraction patterns with fivefold symmetry have been reported from gold, and interpreted as a consequence of twinning on the microscopic scale of the unit cell. But fivefold symmetry should also occur if, for example, metals are deposited from the vapour, when icosahedral packing should be energetically favoured. In November last year, Schechtman *et al.* (*Phys. Rev. Lett.* **53**, 1951; 1984) described the recognition by

means of electron diffraction of icosahedral symmetry in grains of a 14 per cent alloy of Mn in Al, arguing for long-range order of some kind.

This mixture of theorizing and observation has now been put on a broad foundation by D. Levine and P.J. Steinhardt (*Phys. Rev. Lett.* **53**, 2477; 1984) in a summary of largely unpublished theoretical investigations. The objective is to provide a framework for discussing what they call "quasi-crystals".

Again, the starting point is the Penrose tiling of the plane. The outstanding feature of that network is that it lacks translational symmetry, but it has long-range order in that the orientations of the nearest-neighbour bonds (ten altogether, in five oppositely directed pairs) are constant throughout the network. This is the prototype of the quasi-crystal. Bond orientation takes over from translational periodicity.



But how to describe a quasi-crystal analytically? Here is the recipe, for a two-dimensional net. Define a set of unit vectors e_i oriented along the axes of the underlying polygon, where the index i runs from 1 to N , the order of the polygon. Each lattice point can be defined as a linear combination of any two of these vectors, with coefficients x_i , say, but the requirement that the lattice should be a quasi-crystal is that the sequence of projected lattice distances x_i should be the same for all the vectors e_i .

This qualification is not a mere nuisance but the essence of the problem. Levine and Steinhardt conclude that the allowable coordinates for quasi-crystals in two or three dimensions are the positions of lattice points in some one-dimensional quasi-periodic sequence, itself constructed by means of a specific set of rules of which the simplest (leading to the Penrose lattice) is the sequence due to Fibonacci, the thirteenth century Italian who made arabic numerals fashionable in Europe.

The rule is simple. Let there be two intervals of length, r and s , and construct a sequence iteratively, by replacing r by rs and s by r . It is easily seen (by starting with a sequence consisting only of r or s) that the result is the successive addition of new pieces to what is otherwise a steadily lengthening string of intervals, which may be thought of as a one-dimensional lattice (defining the coordinates x_i). The arrangement is plainly not random; pairs of the intervals s never occur in sequence, for example. But it is also not periodic. Moreover, for a sufficiently long sequence, the intervals r and s occur in the ratio $(1 + \sqrt{5})/2$, the golden mean of antiquity which is also one of the solutions of the equation $\tau^2 = \tau + 1$. If the intervals r and s are also in the ratio of the golden mean, the result is a quasi-periodic sequence that defines the Penrose pattern.

Much of the interest in what Levine and Steinhardt have done is their generalization of this procedure. Their schemes for constructing other quasi-periodic sequences for defining lattice coordinates will provoke a search for other kinds of forbidden symmetry. The applications to three dimensions are described only heligraphically, but Levine and Steinhardt appear to have recovered Mackay's result that icosahedral symmetry in three dimensions can be obtained from a set of interpenetrating rhombic tricontrahedra (shapes with 30 faces, 32 vertices and 60 edges). This analytical account should stimulate both the study of other quasi-symmetries and the calculation of their physical properties.

Levine and Steinhardt produce their most startling success in this second connection, with a calculation of the X-ray diffraction pattern from a quasi-periodic icosahedral crystal. That a quasi-crystal should yield diffraction spots is not all that surprising, for the lattice must be built from a small number of recurring intervals. But the big surprise is that Levine and Steinhardt recover almost exactly the pattern found by Schechtman *et al.*

For crystallographers, the implications are far-reaching. There will be a search for other forbidden symmetry. What the new theory suggests for crystal growth is intriguing; which regular lattices most closely correspond to those of which quasi-crystals? What about band-structure in quasi-crystals? Whatever the outcome, crystallographers will have to mug up some unfamiliar mathematics. **John Maddox**

Sensory transduction

New visions in photoreception

from Jennifer Altman

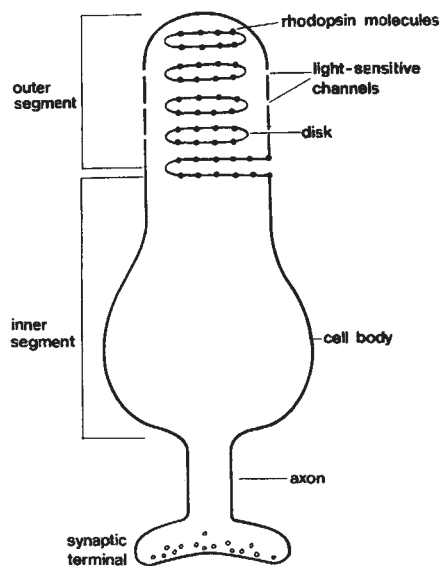
THE testing and disproof of hypotheses is the substance of science but it is not often that we have the opportunity to witness the simultaneous overthrow of a long-standing idea and presentation of evidence that firmly establishes the alternative. Such a treat was the high point of a recent Dahlem conference on the mechanism of photoreception*. Fourteen years after it was first suggested, the hypothesis that calcium is the messenger that couples photoactivation of rhodopsin in photoreceptors of the vertebrate retina to the hyperpolarization of the surface membrane no longer seems tenable. Instead it has been established that cyclic GMP keeps the light-sensitive channels open in the dark and that its light-activated hydrolysis allows the channels to close. The experimental basis for these claims will be presented in a series of papers in this and subsequent issues of *Nature*²⁻⁵. Even more exciting, we are, for the first time, coming close to understanding the molecular mechanism underlying transduction — the conversion of stimulus energy to electrical signal — in any sensory receptor.

The rod photoreceptor, responsible for monochromatic vision in dim light, is a tripartite structure (see figure). The transduction process takes place in the rod outer segment (ROS), which contains membranous disks carrying the photosensitive pigment, rhodopsin. Impinging photons activate rhodopsin molecules and result in a potential change across the ROS plasma membrane. Unlike other excitable cells, rods are active when unstimulated: in the dark, the ion channels in the plasma membrane ('light-sensitive' channels) are open and the continuous inflow of sodium ions gives rise to the 'dark' or photosensitive current^{1,6}, with the consequence that the receptors are depolarized. Illumination causes the channels to close so that the whole receptor (ROS, inner segment and synaptic terminal) hyperpolarizes and transmitter release is reduced.

It has long been accepted that the transduction process requires an internal messenger⁷. As the reduction in the light-sensitive current starts a millisecond after intense illumination, the messenger is likely to be a small, diffusible ion or molecule. One long-held hypothesis has been that calcium is released from the disks by rhodopsin activation and diffuses to the plasma membrane, where it blocks the light-sensitive channels¹. An alternative hypothesis, first proposed about the same time⁸, is that a cyclic nucleotide serves as messenger. Although biochemical analyses long ago showed that light activation of

rods triggers a cascade of enzymatic reactions leading to the hydrolysis of cyclic GMP^{8,9}, this idea has been slow to be accepted because the enzyme reactions have not been considered rapid enough to account for the response.

Evidence in support of the calcium hypothesis was never wholly convincing. Some of the bound calcium in the rods may be released on illumination (although recent measurements make this doubtful¹⁰) and if the calcium concentration inside the rod (Ca_i) is raised artificially, the light-induced hyperpolarization is reduced, which could be due to calcium blocking light-sensitive channels. But the basic requirement of the hypothesis, that light induces a rise in Ca_i , is now a matter of controversy. At the meeting, support for an increase of Ca_i with illumination came from G. Fain (University of California,



Los Angeles), who used microanalysis of shock-frozen, freeze-substituted sections of frog retina¹¹; and from J. Korenbrot (University of California, San Francisco), who measured calcium efflux from intact toad retina with a calcium-sensitive electrode¹². Both these results have recently been challenged^{10,13}, while new experiments reported by Yau and Nakatani^{3,14} suggest strongly that Ca_i falls rapidly on illumination and only recovers in the dark. Recording transmembrane currents from intact, isolated rods, they find that both sodium and calcium enter through the light-sensitive channels in the dark but once the channels are closed there is only a transient efflux of calcium. They interpret this as showing that in the dark a calcium influx through the light-sensitive channels is balanced by efflux through the light-insensitive sodium/calcium exchanger¹⁴,

also located in the plasma membrane. When the light-sensitive channels close on illumination, the calcium influx ceases but the efflux continues for about 0.5 seconds until Ca_i falls so low that the exchanger stops working. Thereafter, Ca_i remains low as long as the light is on. Yau and Nakatani's conclusion is supported by the new measurements of calcium fluxes in the whole retina made by Gold¹³.

The question of Ca_i levels will be resolved only by direct measurements but this presents an extremely difficult technical problem. There is, however, additional evidence that changes in Ca_i levels do not directly affect the photosensitive current. Isolated rods bathed in a solution containing no free calcium still produce photoresponses (U.B. Kaupp, Universität Osnabrück). If Ca_i rises, it apparently does so too slowly to account for the speed with which light-sensitive channels shut (P.A. McNaughton, Cambridge University). Moreover, when calcium buffers such as EGTA or BAPTA, which oppose changes in calcium concentration, are incorporated into isolated rods, the onset and rising phase of the response to bright flashes are not affected (T.D. Lamb, Cambridge University and ref. 4). Taken together these experiments weigh heavily against calcium as the internal messenger.

Several other contenders for the internal messenger have been mooted but only cyclic GMP has received serious recent support (W.H. Miller, Yale Medical School). Miller and Nicol demonstrated in 1979 that cyclic GMP injected into rods causes reversible depolarization, which is antagonized by light¹³, but they did not prove that it was the light-sensitive conductance which was affected. This has now been demonstrated using a patch electrode to incorporate cyclic GMP into whole rods and measuring the current only in the ROS^{4,5}, all of which is light-sensitive⁵. In the dark only about 10 per cent of the light-sensitive channels are open at one time¹⁵, so that the increased depolarization seen when exogenous cyclic GMP is introduced suggests that cyclic GMP is a negative messenger that keeps channels open in the dark. Channel closing probably results from the hydrolysis of cyclic GMP to 5'-GMP by a specific phosphodiesterase, which is turned on by a high-gain chain of reactions starting with photoactivation of rhodopsin and mediated by a guanine-nucleotide binding protein, sometimes called G protein or transducin (see ref. 16).

Attempts to measure changes in the cyclic GMP concentration inside rods with illumination have, however, been equivocal (Miller), possibly because much of the cyclic GMP is bound, leaving only a small active pool (P.J. Stein, Yale Medical School and ref. 17). Cyclic GMP hydrolysis is balanced by resynthesis promoted by guanylate cyclase. Even in the dark this flux is high, equivalent to turning over the whole pool in two seconds. This has led to

*The Molecular Mechanism of Photoreception, Berlin, FRG, 25-30 November 1984.

Ten years of Dahlem Conferences

THE meeting on the molecular mechanism of photoreception was the fortieth workshop run by the Dahlem Conferences and marked the tenth anniversary of the organization with a flourish.

Founded in 1974 by the German Association for the Promotion of Sciences and Humanities in cooperation with the Deutsche Forschungsgemeinschaft, the Dahlem Conferences are financed by the founders, the Senate of the City of Berlin and various commercial interests. They aim to promote the international interdisciplinary exchange of scientific information and ideas, and to stimulate international cooperation in research. Four meetings a year are held in Berlin, usually three on biological topics and one in physical sciences. The areas chosen are usually complex and tend to cut across traditional subject boundaries.

The success of the meeting lies in the format of the workshops, which has been developed, under the leadership of the director, Dr. Silke Bernhard, to ensure a high level of discussion. About 40 participants from all over the world are invited to each meeting, with a number of places reserved for German research students. Background papers are cir-

culated before the meeting so that the four small discussion groups within each workshop can concentrate on areas of uncertainty and identify new questions and directions. The discussions of each group are presented as a summary report at a final meeting of all participants, and then revised in the light of this debate. These reports, together with the background papers, are published rapidly as a book in the series 'Dahlem Workshop Reports'.

Each Workshop lasts a week and gives plenty of opportunity for informal meetings between participants, outside the working sessions. This has facilitated many international and interdisciplinary collaborations and allows people to discuss points of difference in private. As a mark of its success, the 'Dahlem model' has been adopted as a basic format for many other conferences.

The anniversary was celebrated on 12 December 1984 with a seminar for the founders and sponsors, at which six participants of previous Dahlem conferences recounted their impressions of the meetings and described the effects on their subsequent research.

Topics for the 1985 workshops are biotechnology, phanerozoic life and mechanisms of cell injury. Jennifer Altman.

the suggestion that the high energy turnover involved in the flux, rather than absolute concentrations of cyclic GMP, could play the key role in phototransduction (N. Goldberg, University of Minnesota). The alternative, and more likely, view is that the energy stored in the cyclic GMP molecule does not have to be harnessed on hydrolysis but rather ensures that hydrolysis has a high activation threshold and proceeds very rapidly once initiated — both necessary properties for a good switching mechanism. A bucket of water on a high building is a good analogy: stable until pushed off the edge, when it falls rapidly (E. N. Pugh Jr. University of Pennsylvania). In the rod, small local concentrations of cyclic GMP at the channels would be sufficient, and K.-W. Yau (University of Texas, Galveston) gave an estimate of 1–15 μM of free cyclic GMP per rod.

Up to now, however, there has only been speculation about the way that cyclic GMP could control the photosensitive current. The most common model, derived from other cyclic nucleotide messenger systems, involves phosphorylation of channels in the dark to keep them open, using a cyclic GMP-activated kinase, and a phosphatase to dephosphorylate the channels and close them when cyclic GMP levels drop in the light. So far, cyclic GMP-dependent kinases and phosphatases have not been convincingly demonstrated in rods (M.D. Bownds, University of Wisconsin). On theoretical grounds, Pugh argued that a kinase/phosphatase mechanism is unlikely

as it would be energetically too costly, because of the speed of the photoresponse (see also ref. 5).

His argument received experimental support at the end of the last discussion session, when Yau electrified the whole meeting by describing experiments by Fesenko *et al.*² and his own group (unpublished), where bathing the inside of isolated patches of ROS membrane with cyclic GMP produced a rapid, reversible increase in conductance. The effect is not dependent on ATPase or GTPase and occurs in the absence of kinases and phosphatases. Changes in calcium concentration, in the absence of cyclic GMP, have no effect on conductance at all. This is clear evidence that cyclic GMP acts directly on the channels to keep them open, probably by cooperative, allosteric binding. Similar observations of conductance changes controlled directly by cyclic GMP have also been made for disk membranes *in vitro* (ref. 18; Kaupp).

The idea of channels held open in the dark by cyclic GMP fits well with data on channel kinetics¹⁵. The light-sensitive ROS channels are far smaller than any other known channel — they pass an average current of $4 \times 10^{-15} \text{ A}$. In the dark about 10,000 per rod are open at once, with a mean open time of 2 ms. Light reduces the opening rate; that is, once channels close they do not open again, which is consistent with the idea that cyclic GMP is removed by hydrolysis in the light.

Even so, the game is not yet over. Both Matthews *et al.*⁴ and Cobbs and Pugh⁵ find

that exogenous cyclic GMP does not slow the rising phase and prolongs the duration of the photoresponse, both inconsistent with the idea that a simple binding of cyclic GMP keeps channels open. Although the allosteric binding reported by Fesenko *et al.*² goes some way to resolving this anomaly, theoretical analysis indicates that the free cyclic GMP would have to be much lower than published estimates and that the bound cyclic GMP must be inexchangeable on the time scale of the rising phase of the photoresponse⁵. Moreover, although it is no longer creditable as the messenger, calcium seems to play some role since Ca_i in the rod is carefully regulated³ — it could be involved in adaptation of the light response by modulating enzymic activities. More intriguing still is the recent discovery of inositol triphosphate (ins P_3) in vertebrate photoreceptors and the possibility that there are branched pathways in the transduction mechanism (J.E. Brown, Washington University, St. Louis), particularly as Ins P_3 seems to be involved in releasing bound calcium in many cells¹⁹. So although the results of Fesenko *et al.* and Yau are a breakthrough in understanding phototransduction in vertebrate rods, clearly there are still baroque complexities to be unravelled.

Are these features specializations of photoreceptors, or could they have far-reaching implications? This is the first time that a cyclic nucleotide has been shown to regulate any channel directly, rather than through protein phosphorylation; are other examples waiting to be found? Are the tiny light-sensitive channels, carrying currents that are within the noise level of patch clamp recordings, unique to the ROS, or could they be lurking among the larger channels in other excitable membranes? The highly sophisticated techniques needed to work on tiny photoreceptor cells are providing new challenges to those working on ostensibly more straight-forward systems. □

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Volcanology

Volcanic gas release rates

from Lionel Wilson

BECAUSE compounds are generally much more soluble in magmas at the high pressures of their mantle source regions than at the surface, volcanoes generally release gases. But the rates of gas release are difficult to quantify. Thanks, however, to the excellent long-term monitoring programme of the US Geological Survey's Hawaiian Volcano Observatory, T.M. Gerlach and E.J. Graeber of the Sandia National Laboratories have been able to estimate the volatile budget of Kilauea, most active of the Hawaiian volcanoes, over the past 27 years. Their results are published on page 273 of this issue.

Like many volcanoes, Kilauea has a high-level magma storage zone, consisting of a network of dykes and sills. Parental basaltic magma, rising at a fairly constant rate from deep mantle sources, is either erupted directly to the summit area or stored causing inflation of the volcanic edifice. If direct eruption takes place, as commonly occurred before 1924, the released gases will be typical of the volatile content of the source regions at depth. Gerlach and Graeber, who call these volatiles Type I gases, suggest that a sample of such gases has only once been collected without significant contamination by air or atmospheric water (by J.A. Jaggar in 1917-18). This sample contained about 59% carbon dioxide, 18% water vapour and 10% sulphur (as sulphur dioxide) by weight, with traces of carbon monoxide, hydrogen, hydrogen sulphide and hydrogen chloride.

Since 1924, direct eruption of parental magma at Kilauea has been rare; instead, magma is stored in the summit reservoir, exsolving volatiles until equilibrium is reached with the local pressure. Presumably these excess volatiles either leak to the surface or become involved in chemical reactions, perhaps in near-surface hydrothermal systems. Intermittently, magma migrates sideways into the east or south-west rift zones to erupt at distances of up to tens of kilometres from the summit. The amounts of volatiles (termed Type II gases) released at the vent represent the difference between the equilibrium states in the storage zone and at the surface. Samples of these gases (collected by several scientists in recent years) consist of about 69% water vapour, 22% sulphur (mainly as sulphur dioxide) and 8% carbon dioxide by weight, with small amounts of hydrochloric acid, hydrogen fluoride, hydrogen and traces of atmospheric gases.

Knowing the relative amounts of these volatile compounds in Types I and II gases is, however, only the first stage of establishing the absolute amounts released by the erupting magma. The key link comes from

a comparison of the sulphur contents of three types of erupted material: samples of spatter from vents on land; samples of lava erupted underwater from vents forming an off-shore extension of the east rift zone; and glass inclusions in olivine crystals forming part of the erupted magma.

The spatter samples represent droplets of magma which have had ample opportunity to release all their excess volatiles while forming part of a fire-fountain above the vent. The submarine lavas, on the other hand, have been subjected to water pressures high enough to cause them to retain essentially all of the volatiles present in the storage zone. Finally, the glasses trapped inside olivine crystals should have had no opportunity to release any volatiles since leaving the parental magma source in the mantle. Thus, the difference between the sulphur contents of the spatter samples and the submarine lavas should give the absolute weight fraction of the sulphur lost by the magma into Type II gases, and the difference between the sulphur contents of spatter samples and glass inclusions should give the absolute amount of sulphur lost to Type I gases. It is then a simple matter to convert the ratios of the amounts (relative to sulphur) of the other compounds that are present in the gases to their absolute weight fractions.

From the well-documented volumes of lava erupted from Kilauea in Type I and Type II activity since 1956, Gerlach and Graeber calculate a total volatile budget which shows that, on average, anthropo-

genic inputs of carbon dioxide and sulphur dioxide to the atmosphere exceed those of the volcano by factors of 15,000 and 380, respectively. It would, of course, be very valuable to have the same kind of information for other commonly-active volcanic centres, so that a global budget could be calculated, but several factors conspire to make this difficult. First, the physical collection of uncontaminated gas samples near volcanic vents is never easy; remote-sensing methods of gas analysis using spectroscopic methods are effective but have difficulty dealing with the minor components of the mixture. Second, whereas Kilauea fortuitously provides lava samples in submarine eruptions which are typical of the magma in high-level storage, in general such material is not available. (The alternative technique of analysing glass inclusion in magmatic crystals for evidence of the pre-eruption magma chemistry is becoming accepted as reliable, but there is sometimes considerable ambiguity as to the depth in the volcanic system at which the entrapment took place.) Third, the Hawaiian Volcano Observatory monitors the volumetric output of Kilauea with a thoroughness that is seldom matched elsewhere; and, finally, many volcanoes show a greater variation of magma chemistry with time than does Kilauea, so that it would require reliable data from every eruption for calculation of an accurate budget.

Gerlach and Graeber's work has underlined what can be done by careful study. Only with strong financial support for volcano monitoring will it be possible to carry out this kind of analysis of other active centres. □

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Psychophysics

Structured representation in low-level vision

from R. J. Watt

BEFORE we can claim to understand vision, we must provide explanations of the ability to discriminate and the appearance of visual stimuli that go beyond being simply descriptions of the stimuli themselves. At the centre of our gaze (the fovea), vision is usually so faithful to the stimuli that its operations are well concealed. But on page 308 of this issue Rentscher and Treutwein show that this is not the case outside the fovea, where, it seems, much less powerful representations are constructed¹.

Rentscher and Treutwein have compared the abilities of foveal and peripheral vision instantly to discriminate pairs of different spatially periodic stimuli. Specifically, the stimuli are constructed by superimposing a cosine wave grating with its third

harmonic, as shown in the figure. For each pair of stimuli, the relative phases of the fundamental and third harmonic differ by 120° around a mean phase difference of 0°, 90° or 130° (so the relative phases of the pairs are: -60°, +60°; +30°, +150°; +120°, +240°). Only the pair around 90° differ in contrast profile; the other two pairs are mirror images of each other. Although each pair is discriminated equally well when seen in foveal vision, in peripheral vision the 90° pair is discriminated much more easily than the mirror-symmetrical pairs.

Rentscher and Treutwein interpret this result as implying that the relative position of local image components or features is lost in peripheral, but not in foveal vision.

Since the two cosines themselves cannot be the components in question, what are? Inspection of the stimuli (see their Fig. 1) suggests, however, that those around 0° (the top pair and that on the left of pair b) have a different type of appearance from the other three, which are around 180° . In the former three, one sees broad dark and light bars of roughly equal width with finer detail superimposed; in the latter, the broad dark band is much wider (or much narrower, depending on what you consider to be its edges). One might speculate that in pair b there is an immediate difference of organization or grouping of the stripes between the two patterns, whereas in pairs a and c scrutiny is required to spot the differences in detail. The position of each stripe is thus coded in two ways: by the group to which it belongs and by its position within a group. This elaborates the experimental results, and one might wonder

been that there are two 'phase-sensitive devices' which categorize all phase relations between different channel images into 0° or 180° angles³. Discrimination of phase angles either side of the category boundary ($\pm 90^\circ$) would be good, but discrimination of phase angles symmetrically placed either side of the category centre (0° or 180°) would be impossible. Whilst this matches the extrafoveal visual performance, one would have to invoke a wholly different mechanism for the fovea, where phase discrimination does not markedly vary with mean phase⁴. The suggestion that two regions of retinal space separated by so small a distance should be subject to such different types of representation (such as two-state and continuous), is unreasonable.

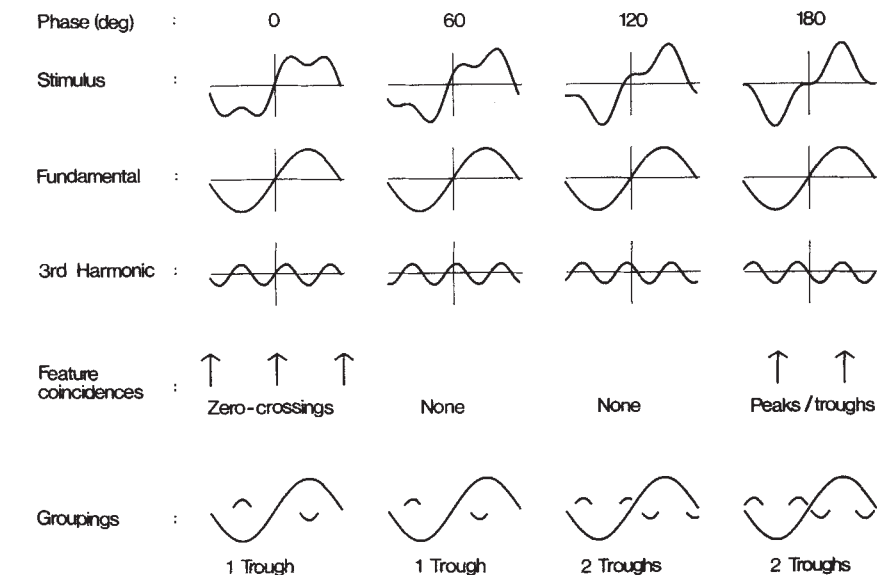
A very similar suggestion arises (for different reasons) from the theory of edge detection of Marr and Hildreth⁵ who suggested that the places where the image of

description of the phenomenon where I was suggesting that the elements of the representation correspond to the stripes of the patterns. This is not to say that they form an unstructured list, recording quite independently the location, brightness and so on of each stripe, with refinements being appended to each group to further distinguish the elements of the group. For example, the bottom pair of the figure shows how the half-waves of the fundamental can group together some of the troughs of the third harmonic so that the second half of each cycle in the figure is represented as a broad bright bar plus one or two small darker bars. This representation would be sufficient to allow a discrimination between the $+30^\circ$ and $+150^\circ$ patterns but not the others. A more refined representation would additionally note the relative positions of the small bars. Then, to explain the observations, one simply has to suppose that the refinements necessary to discriminate between the -60° and $+60^\circ$ patterns are too subtle for the representational accuracy of extrafoveal vision, but not for foveal vision. To substantiate this suggestion, readers could experiment with stripe patterns of their own design. All they need to know is that reversing the order of the stripes (which could simply be black lines of differing thickness) negates the phase angles. It will be appreciated that discriminability varies according to how large (and hence how accurately seen) the differences in line thickness are.

Much of this would be wild speculation were it not for the fact that ideas of a related nature have already been mooted. Recently, Julesz⁷ has argued along similar lines for a distinction between the representations formed in preattentive and attentive vision. Using stimuli in which a particular small sub-pattern is repeated many times over a large area, thereby creating a visual texture but in which a small patch has a different sub-pattern, Julesz found that sometimes the odd patch stands out immediately, and sometimes it does not, depending on the particular sub-patterns. The implication is that the immediate, preattentive representation is less refined than that built up through detailed scrutiny of the sub-patterns. Julesz suggests that the process of grouping (and its corollary, segregation) occurs pre-attentively and that further refinement (by detailed scrutiny) requires focal attention. The parallels between the Julesz conjecture and my line of reasoning should be apparent. $\square$

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A selection of the Rentschler and Treutwein stimuli is illustrated by showing: in the top row, the actual stimuli; in the second row, the fundamental cosine wave; and the third row the 3rd harmonic. The various curves plot luminance as a function of space. The position of the third harmonic varies along the row, and the effect of relative position or phase on the spatial coincidence of certain features, such as zero crossings, is shown by arrows. In the bottom row, a way in which the fundamental component might group together the peaks of the third harmonic is suggested. The number of troughs (dark bars) of the third harmonic straddled or grouped by the peaks (bright bars) of the fundamental, changes at a phase angle of 90° and is thus a cue at that point. Elsewhere, the position of the peaks within the grouping would be needed as a cue.

whether the deficit in extra-foveal vision concerns the handling of detailed position in each group.

The early stages of vision are generally regarded as a number of parallel spatial filters or channels, each tuned to a particular band of spatial frequencies² and carrying an image of the stimulus information within that band. The images in the various channels have to be combined to form a composite representation and this is where the results of Rentschler and Treutwein are most interesting because the two cosine sub-patterns in their stimuli will be carried largely by different channels.

One suggested explanation for the segregation of the phase cycle into two distinct patterns of perceptual organization has

each channel crosses zero are recorded. If zero-crossings, as these features are called, are found at the same spatial position in different frequency channels' images then they might be supposed to have a single edge, or step rise in luminance, as common stimulus source. In essence, this proposition — the spatial coincidence assumption — is the same as a 0° phase-sensitive device. Had Marr and Hildreth used the same argument for the places where the channel image reaches a local peak or trough, as many would now do⁶, they would also have had the 180° device. Therefore this edge detection theory is also unreasonable as a model for human vision in the light of the experimental findings.

Let us return to my (heavily interpretive)

Drosophila development

Compartment genes in hand

from Peter A. Lawrence

THE history of *Drosophila* genetics is long and full of happenstance. Typically, mutations were chanced on in one decade, mapped immediately, written down, written up and written off — unless they became useful in mapping other things in later decades. Analysis of the normal function of the gene that carries the mutation was usually not attempted or even thought of, and only recently has it become common practice to use systematic mutagenesis to find out whether the original mutation is typical of mutations in the gene or a freak. A good example is provided by the history of the *engrailed* gene; papers appearing in this issue of *Nature*¹ and in the current issue of *Cell*^{2,3} take the gene into the era of molecular cloning.

The *engrailed*¹ (*en*¹) mutation was reported in 1929 (see figure) but it took more than 40 years for the gene to be recognized as rather special. That story began when Ed Lewis pointed out to Antonio Garcia-Bellido that the posterior edge of the wing of flies carrying the *en*¹ mutation is transformed into an anterior margin. Following Tokunaga's study of the leg⁴, Garcia-Bellido and Santamaria⁵, using mosaic flies, showed that *en*¹ cells affect posterior but not anterior margins of the wing. A year later Garcia-Bellido, Ripoll and Morata discovered that the wing is made in two precise compartments, each being constructed of all the descendants of

a small founding group of cells⁶. They suggested the groups are distinct because they express, and are subject to, a set of regulatory genes, called 'selector genes'⁷. This key idea provided a link between genetics and pattern formation, and prompted a search for such genes, of which *engrailed* was suggested to be one.

Morata and I tested this idea and showed that *en*¹ has no detectable effect on any cell in the anterior wing compartment, but seems to alter every cell in the posterior compartment. We also suggested that the *engrailed* gene 'labels' posterior cells so that they do not mingle freely with anterior cells^{8,9} — the idea being that the compartment border at the interface between anterior and posterior cells is straight because (like oil and water) the different cell populations keep their contact to a minimum¹⁰. The *engrailed* gene is therefore presumed to be responsible for the compartment border itself. We also suggested that when the gene is first activated in development, the patches of active cells need not be arranged very precisely, because uneven lines would automatically straighten as development proceeds¹¹. The *engrailed* gene therefore became a keystone in the compartment hypothesis and has remained an ever-present and enigmatic lodger in our lives.

About ten years ago, Thomas Kornberg began to study the gene. He made the first lethal allele of *engrailed* (*en*^{C2}) in

Cambridge and then made a thorough genetic analysis of the locus, collecting a large number of mutants and studying their embryonic phenotype¹². It was shown that cells homozygous for a mutation that is lethal to the fly are nonetheless perfectly normal when experimentally placed in any anterior compartment (not just the wing) of a heterozygote fly but abnormal in posterior compartments^{12,13}. These and several other studies^{14,16} supported our view that *engrailed* is a selector gene largely or entirely responsible for the differences between anterior and posterior compartments.

The molecular biology of the *engrailed* gene has been pursued for several years by Kornberg, O'Farrell and their colleagues. But it is the recent discovery of the homoeo box by the groups of Walter Gehring in Basel and Matthew Scott in Boulder that has opened up an effective route to the cloning of selector genes. Fjose, McGinnis and Gehring have now followed this route to the *engrailed* gene¹. And Kornberg's group has obtained the same gene by more traditional means^{2,3}. Even more pleasing is what both groups have been able to show by virtue of having isolated the gene.

First, using the *in situ* methods developed for *Drosophila* by Akan¹⁷ and by Hafen *et al.*¹⁸ they have shown that *engrailed* is active in the embryo from early gastrulation onwards and is expressed in the form of circumferential stripes. Both groups have assumed, but not demonstrated, that the active bands coincide with the posterior compartments (Kornberg and colleagues show that the gene is transcribed in posterior compartments later on — for example in the wing disk). Both groups show pictures of the stripes in the main body of the embryo. The total number of stripes is not clear (nor, because frozen sections spoil the anatomy of the older embryos, is the precise identity of the transcribing cells known) but, nevertheless, both groups count 14 stripes in the main body of the embryo, which is exactly the number of metameric units counted by Poulson many years ago¹⁹. (I write metameric units because probably these cell masses are not segments as Poulson thought, but "parasegments" — units consisting of a posterior compartment of one segment plus an anterior compartment of the next²⁰.)

Both groups show that the stripes in which *engrailed* is outlined are about half as wide as those in which it is not, which suggests that the posterior compartments are smaller than the anterior ones. This is shown directly for the first time. (Indirect estimates based on clone frequency and gynandromorphs had shown that there are more anterior precursor cells than posterior ones².) Activity of the *engrailed* gene can first be detected at gastrulation, at which time Kornberg *et al.* show that the posterior stripe is only 1–2 cells wide. If we are correct that *engrailed* product is crucial

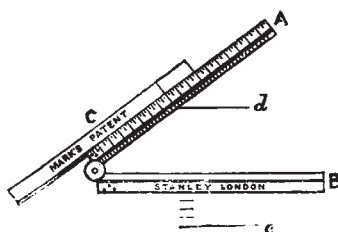


100 years ago A LINE-DIVIDER

GALILEO'S proportional compasses are said to date from the year 1597. We infer that the instrument consisted of two arms, jointed as in the accompanying figure, so that one arm could move freely about the joint. Each arm had a number of equal divisions (not necessarily of the same length on each arm), the zero point being at the joint. To divide a given length into five equal parts is it necessary to take an ordinary pair of compasses and measure the given length with these, then set the proportional compasses so that the fifth division on each arm may be at the given distance apart, then transfer with the ordinary compasses the distance between the unit divisions—this will be one-fifth of the given line. This seems to have been the manner of using the instrument employed by Galileo (cf. Marie, *Histoire des Sciences Mathématiques et Physiques*, tome iii. p. 108). Other modes of using will doubtless occur to most of our readers. The principal involved in this and similar instruments, and certainly in the one before us, is that

of the proportionality of corresponding sides in similar triangles.

Our figures represents Miss Marks's patent line-divider for dividing and spaces into a number of equal parts. A B forms a hinged rule with a firm joint; each limb is ten inches in length (in the specimen we are describing), the limb B is bevelled, fronted with brass, and presents a straight edge, so that straight lines can be drawn along it. The limb A is also bevelled, and is divided on the bevelled edge and also on the top



into eighty equal parts, so that we are enabled to divide a given length into any number of equal parts from two to eighty. A is fitted to slide in an undercut groove upon the plain rule C, which has a single line marked upon it, and is also provided with needle points on the underside, to prevent it from slipping when placed in any position.

From *Nature* 31 275, 22 January 1885.

for the establishment of the anterior and posterior compartments, then its absence at the earlier blastoderm stage suggests that compartments are not founded by then. Probably the parasegments (seen as the stripes of activity of the *ftz* gene at blastoderm^{20,22}) do not become divided up until after the *engrailed* gene is transcribed at early gastrulation. The segment boundary would be established at that time²⁰, which would fit with Kornberg's observation¹² that the lethal mutations of *engrailed* do not show up for about 6 hours after fertilization. Because *engrailed* seems not to be required in muscles and other mesodermal structures, and because the muscles of each segment are not divided into anterior and posterior compartments by lineage, we had suggested that *engrailed* has no role in the mesoderm^{23,24}. This view is supported by evidence from Kornberg *et al.* that transcription of *engrailed* is largely confined to part of the ectoderm. Surprisingly, they find no *engrailed* transcription in the embryonic nervous system — which belongs to the ectoderm and so would be expected to behave like the epidermis although, as they point out, this might be explained if the probe used does not detect all the *engrailed* transcripts.

Both groups report that the homoeo box of the *engrailed* gene has diverged from the other homoeo box sequences and is broken by an intron. Kornberg's group has sequenced an open reading frame of 1,700 base pairs that is upstream of the homeo box. In addition, within a few thousand bases of *engrailed*, they have found another gene that contains an *engrailed*-like homoeo box, has considerable

homology with *engrailed* and is transcribed. What function it subserves is intriguing. So is the observation that the *engrailed* gene protein contains stretches of polyglutamine, polyserine and polyalanine³, and the presence of another homoeo box sequence (quixotically called the 'antibox'), which is on the opposite strand from the *engrailed* gene and may not be transcribed.

The value of using both genetics and cytogenetics to clone carefully-chosen genes has been reemphasized by these studies. So has the value of the *in situ* technique, which provides a meeting place for morphology and molecular biology. Together these approaches allow a fresh onslaught on the ancient problems of embryology and have already led to newer and sharper questions — about segmentation for instance. The *engrailed* experiments leave the compartment hypothesis alive and well and living not only in Madrid. □

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Trace elements

Stable forms of methylated germanium in seawater

from J.D. Burton

In 1981, Froelich and Andreae produced the first adequate view of the geochemical behaviour of inorganic germanium in the oceanic water column¹. The results indicated that germanium follows very closely the cycle of silicon in its uptake into, and subsequent dissolution from, the external opaline shells of organisms. As a result, dissolved inorganic germanium, thought to be present mainly as uncharged $\text{Ge}(\text{OH})_4$, is distributed much like a nutrient, being depleted in surface waters and increasing in concentration with depth; in the deep North Pacific, the concentration reaches about 115 pmol l^{-1} . New findings from the same laboratory, published on page 303 of this issue², show that this intensive internal oceanic cycling of inorganic germanium takes place against a background of uniform high concentrations of methylated forms of the metal, a situation which is unknown for any other element.

The precise measurements of methylated germanium made by Lewis *et al.* emphasize the capacity of marine chemists to determine an increasingly wide range of chemical species at ultra-trace concentrations. The analyses involve reduction of the germanium species to the corresponding hydrides, stripping and cryogenic trapping of the volatile hydrides, release from the trap by heating, and measurement of nanogramme quantities of germanium by atomic absorption spectrophotometry using electrothermal atomization. In this way monomethylgermanium (MMGe) and dimethylgermanium (DMGe) were detected but no trimethylgermanium (TMGe)

could be found.

At various depths, in four widely spaced oceanic locations, the concentration of MMGe and DMGe are close to average values of 326 and 97 pmol l^{-1} , respectively. The small variations that do occur can be accounted for by the same factors that alter the total salt content of seawater in different parts of the ocean. This conservative behaviour, which is shown by only a few among the minor oceanic constituents, is indicative of a very low geochemical reactivity. Measurements on anoxic waters indicate that the lack of reactivity extends to reducing conditions. Concentrations of MMGe and DMGe in various river waters are lower than those in seawater by factors of about 100 and 10, respectively. Changes of concentrations in estuaries, which are often highly reactive geochemical systems, reflect simple dilution, further emphasizing the remarkably unreactive nature of the methylated species.

This picture of methylated germaniums as stable, uniformly-distributed species contrasts strongly with the highly dynamic interconversions of other methylated metals. The data have largely emerged since it was recognized, in the late 1960s, that methylated species play an important role in the natural aquatic environmental behaviour of mercury. Methylated species of antimony, arsenic and tin are present in natural waters (see, for example, ref.3) and there is evidence that other metals can undergo methylation under environmental conditions. The concentrations of methylated species in seawater reflect a transient

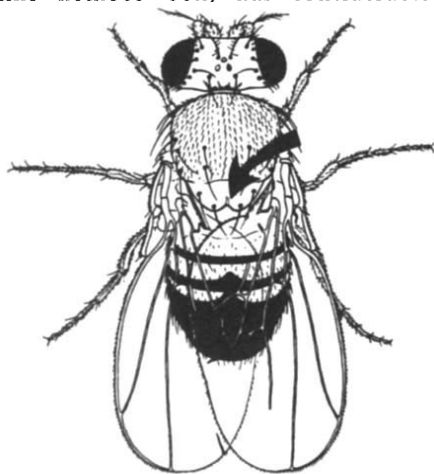


Fig. 1. *Engrailed* male.

"Nov. 7, 1926 seven males and eight females, which had a longitudinal cleft along the middle of the scutellum and irregular venation of the wings, were found by K. EVANG in the vesiculated stock" is the opening sentence of Reider Eker's original designation of the 'engrailed scutellum' (now *engrailed*) mutant of *Drosophila*. Although the cleft often divides the scutellum in two, "not infrequently it is restricted to a small median notch at the hind border of the scutellum, thus giving this hind border an 'engrailed' appearance." The figure is taken from Eker's paper in *Hereditas* **12**, 217; 1929 but the arrow to the notch has been added.

balance between their formation, transport, and removal, including decomposition. This is best documented in the case of arsenic. The occurrence of monomethylarsonic acid and dimethylarsinic acid is variable and largely limited to the euphotic zone, where they form a significant but not dominant fraction of the total dissolved arsenic⁴. Marine algae in culture produce methylated forms of arsenic from arsenate^{5,6}, while bacterial cultures isolated from estuarine waters demethylate dimethylarsinic acid⁷.

The sources of methylgermanium species are not yet known. If they are produced largely on land and carried to the oceans by rivers, the results suggest mean oceanic residence times of about 4 million years for MMGe and half a million years for DMGe, values which are comparable with the residence time of, for example, calcium. On the basis of its close geochemical coherence with silicon, the residence time of inorganic germanium is probably about 10,000 years. The apparent, radiocarbon-based age of 3400 years for total dissolved organic matter in some of the oldest waters in the general oceanic circulation⁸, cannot give unequivocal life-times for particular organic species since dated material is a mixture of compounds that are decomposed or otherwise removed at different rates. Thus the unpredicted existence of very low concentrations of organic species with residence times of about a million years is not necessarily inconsistent with other findings.

The reasons for the stability of MMGe and DMGe remain to be ascertained. The

experiments of Lewis *et al.* on the effects of photo-oxidation with ultraviolet radiation which raise further questions in this respect. They show that methylated germanium species are not decomposed by ultraviolet radiation in natural sea water but are totally decomposed in pure water. In artificial seawater, partial decomposition of DMGe and TMGe to MMGe proceeds more readily than conversion to inorganic germanium. Micro-organisms are often able to carry out reactions that are difficult to achieve by chemical or physical means in the laboratory, and they need to be considered if the reasons for the environmental stability of MMGe and DMGe are to be understood.

In showing distinctive and unexpected biogeochemical behaviour in the marine environment, germanium belies, in certain respects, its old title of ekasilicon. The element is now seen to have an interesting part in the unfolding story of environmental methylation of metals. □

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Atmospheric chemistry

Mesospheric ozone variations caused by gravity waves

from Guy Brasseur

FOR about three years, the Solar Mesosphere Explorer satellite has been measuring the key parameters for understanding the budget of ozone in the middle atmosphere (mesosphere). As I have already explained in these columns (*Nature* 305, 15; 1983), in addition to continuous observations of ozone by an ultraviolet and an infrared spectrometer, the satellite provides data on the temperature, the concentration of nitric oxide and nitrogen dioxide, and the strength of the ultraviolet solar irradiance in the mesosphere. A recently published analysis by R.J. Thomas and his collaborators (*Geophys. Res. Lett.* 11, 673; 1984) of the 1982 and 1983 data establishes new and interesting features.

In particular, it has been established that there are large seasonal variations in the ozone density at an altitude of about 80 km and that these variations are highly reproducible from year to year in both hemispheres. At this altitude the ozone concentration exhibits a so-called secondary maxi-

mum as is known both from rocket experiments and from numerical models. This concentration is of the order of 10^8 molecules cm^{-3} , which is 4 orders of magnitude smaller than the well known maximum at 25 km altitude, and results from complex chemical reactions involving oxygen and hydrogen components, and from dynamical processes which are responsible for the transport of long-lived species such as water vapour or nitric oxide. The detailed analysis of the satellite data on the secondary maximum concentrations show that the peak concentration at the equinoxes is about 2 to 3 times greater than at the solstices. The data at 45°N latitude and 0.01 mbar (about 80 km altitude) show that the maximum concentration occurred in spring on April 22 in both 1982 and 1983 and that in both years there was a smaller peak at the end of September. The southern hemisphere's data show the same pattern but with a time-lag; at 45°S, the large peak is in the middle of October and the small peak

at the end of March.

These seasonal changes cannot be explained solely by photochemical considerations since the solar input, and consequently the ozone production and concentration, exhibit a maximum at the summer solstice and not at the equinox. Thomas and his colleagues suggest that the changes are also related to the variation of the transport strength induced by the dissipation of gravity waves.

Gravity waves, which are perhaps most familiar as the waves that propagate on the surface of water, are also present in a stably-stratified atmosphere, where they propagate both horizontally and vertically. They are produced in the troposphere (the first 12 km or so above the Earth's surface) by a variety of mechanisms including air-flow over surface topographic features. Since, in the absence of dissipation, the kinetic energy density must remain constant, the amplitude of the gravity waves will grow with altitude as the inverse square of the air density. When the density of the temperature wave associated with the density wave becomes large enough to produce a local superadiabatic lapse rate, the wave becomes unstable and dissipates: the breaking of the wave may be an important source of local energy and produces turbulent diffusion. In other words, the process of wave breaking controls part of the transport of chemical constituents in the mesosphere.

The vertical propagation of gravity waves is directly dependent on the zonal wind structure in the atmosphere. In winter, when the zonal winds are westerly, the waves with easterly phase speed reach the 80 km level; in summer, when the mean zonal winds are reversed, it is the westerly phase speed waves that reach the mesosphere. In early spring, however, the winds are westerly in the stratosphere but easterly in the mesosphere and the waves are so rapidly absorbed that no dissipation occurs. Turbulent diffusion in the mesosphere is therefore considerably reduced near the equinoxes. Consequently, the water vapour near 80 km, which is transported from lower levels, is less abundant in spring and autumn than at other periods; since water molecules seem to be the most active agent of ozone destruction in the mesosphere, one should expect slower rates of destruction, and consequently larger ozone concentrations, at the period when the zonal winds reverse. Thomas and his colleagues therefore suggest that variations in the ozone density may provide a sensitive indicator of the effects of gravity waves on the middle atmosphere. Further analysis of the Solar Mesosphere Explorer's ozone measurements, which represent the largest climatological data base of ozone ever compiled, should continue to produce rich rewards. □

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Yellow rain and the bee faeces theory

SIR — Two different inferences may be made from the observations reported by Nowicke and Meselson in "Yellow rain — a palynological analysis"¹. (1) That yellow rain samples (claimed by the US government to be evidence of chemical warfare²) are nothing more than bee faeces and therefore the findings^{3,4} of trichothecene mycotoxins in some of the samples collected in both Laos and Kampuchea resulted from colonization of the faeces by trichothecene-producing *Fusarium* spp. Or (2), some of the chemical warfare samples became contaminated by bee faeces as a result of bee activity in the battle areas during the period between chemical attack and sample collection or because the chemical warfare agents had landed on previously deposited bee faeces.

The first inference is a prime basis for the so-called bee faeces theory. Since so much attention has been given to this theory, it is of some importance that the theory be scrutinized in light of what is known about trichothecene production from laboratory experiments as well as reported natural occurrences.

First, for *Fusarium* spp. to produce high levels of toxins on any particular substrate, the substrate must be heavily invaded by fungi. Bee faeces that had experienced such an invasion would no longer be yellow, but would have an off-white colour characteristic of fungal mycelia. In addition, the material would be fluffy or matted rather than powdery, a reflection of the morphology of the mycelium rather than of the pollen. Those of us who analysed the chemical warfare samples analysed yellow powders and green-brown leaves.

Second, those *Fusarium* spp. that produce Type A trichothecenes such as T-2 toxin or diacetoxyscirpenol (DAS) only rarely produce Type B toxins such as nivalenol (NIV) or deoxynivalenol (DON) and vice versa⁵. These laboratory findings are supported by data from numerous analyses of foods and feeds involved in natural outbreaks where Type A and B trichothecenes have never (except in one case) been found together⁵. The lone exception was corn from France⁶ contaminated with 4.28, 0.6 and 0.02 $\mu\text{g g}^{-1}$ NIV, DON and T-2 toxin, respectively. These results were never confirmed by techniques such as mass spectrometry nor is it easy to measure so little T-2 toxin by thin-layer chromatography with any confidence. Canadian investigators⁷, using a "multi-residue" detection method based on selected ion monitoring, found 36 wheat samples contaminated with DON; not one of these samples contained T-2 toxin or DAS. In contrast, high levels of T-2 toxin, NIV and DON were found in vegetation samples collected in Kampuchea⁴ and high levels of T-2 toxin, DAS and DON were found in a powder collected in Laos³. While it is possible that more than one *Fusarium* spp. might have colonized these

samples, it has yet to be demonstrated that such an event will result in the formation of high concentrations of both Type A and B trichothecenes in nature. It is interesting that the Laotian powder contained essentially equal concentrations of T-2 toxin, DAS and DON, a mixture found to have a synergistic effect on mouse LD₅₀ after topical application⁸.

A third problem with the bee faeces theory is the failure to detect HT-2 toxin in any of the chemical warfare samples. Surely, one would expect bee faeces to contain significant amounts of microbial esterases⁵, capable of transforming T-2 toxin into its de-acetylation product, HT-2 toxin. The counter-argument — that there is insufficient moisture in bee faeces to support bacterial growth — runs into the question of insufficient moisture in bee faeces or on leaf and rock surfaces to support the growth of *Fusarium* spp. Critical moisture content levels for development of *Fusarium* spp. vary between 22.2 and 30.0% in cereals⁹.

Fourth, the absence of HT-2 toxin in the chemical warfare samples is not in accord with a natural explanation because laboratory cultures of *Fusarium* spp. produce HT-2 toxin at temperatures common to South-East Asia¹⁰.

Fifth, while faeces can support the growth of some fungi, it has not been established that *Fusarium* spp. can grow on faeces, let alone produce secondary metabolites such as mycotoxins on this substrate in the high concentrations reported. In fact, US Army analysts have failed to detect any trichothecenes in numerous vegetation samples contaminated with "yellow spots". It is also well established that *Fusarium* spp. prefer either the delicate tissues of rootlets and seedling stems or senescent tissue of plant origin¹¹.

Sixth, recent studies¹² have shown that trichothecenes are not produced on unwashed and unsterilized pollen, even under controlled laboratory conditions. *Fusarium* is not a good competitor in colonizing organic matter such as pollen and will be outgrown by more aggressive fungi such as *Penicillium* and *Aspergillus*. Furthermore, two species of *Fusarium* grown on washed and autoclaved rice and pollen produced zearalenone on the rice but not on the pollen, a result at variance with the findings of high zearalenone concentrations in two of the chemical warfare samples^{3,4}.

Seventh, again under controlled laboratory conditions, only one of thirteen *Fusarium* spp. isolates collected in Thailand was capable of producing trichothecenes, and this in low yield¹³.

Finally, the presence of polyethylene glycol in a powder collected in Laos^{3,14} has never been dealt with, except for unsubstantiated allegations of sample or laboratory contamination¹⁵.

To summarize, the bee faeces theory does not adequately explain the findings of unusually high concentrations of trichothecenes and zearalenone in very unusual combinations produced on a moisture-deficient substrate under climatic conditions favourable for the production of deacetylated metabolites.

The bee faeces theory, to the best of our knowledge, had its genesis in samples collected after an unexplained dropping of yellow powder on a Thai village near the Kampuchean border by an unidentified aircraft. The theory gained credence because two samples that were positive in tests for trichothecenes were contaminated with pollen. In retrospect, this is not too surprising, since these samples were collected during the pollinating season and the samples could have become contaminated with pollen before or after a yellow rain attack. The theory gained further credence when people indigenous to the area collected and turned in anything that was yellow, in spite of the fact that yellow rain attacks were rare by mid-1982. About eighteen months have passed since the bee faeces theory was first publicly proposed¹⁶, and we are amazed that the proponents of this theory have not yet presented one iota of evidence that trichothecenes can be produced naturally on bee faeces, rocks, leaves, gas masks and so on. The only evidence presented so far is that bees defaecate. We can only ask, "so what?"

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Climatic effects of Volcanic eruptions

SIR — The appropriateness of using volcanic eruptions as a basis for estimating the climatic effects of nuclear war has recently been discussed^{1–5}. Generally speaking, the relatively small quantities of aerosols produced by historic eruptions and the differences in the optical properties between volcanic aerosols (H_2SO_4 and fine ash) and the sooty smoke from fires generated by nuclear blasts make any comparisons between the atmospheric after-effects of historic eruptions and nuclear war tenuous^{1,2}. In other instances, namely those of the largest-scale eruptions, volcanic analogies may be relevant.

The greatest known Quaternary explosive eruption was the Toba (Indonesia) outburst of 75,000 years ago, which erupted approximately $1,000 \text{ km}^3$ of rhyodacitic magma in perhaps 9 to 14 days^{6,7}. The widespread Toba ash layer is found over at least $5 \times 10^6 \text{ km}^2$ (ref. 6), but the total amount of long-lived stratospheric H_2SO_4 aerosols produced by this eruption is not known. We may, however, make some estimate of this quantity by using data from more recent eruptions of comparable magma type⁸. The sulphuric acid aerosols generated by the small 1980 Mt St Helens eruption, which produced 0.35 km^3 of dacitic magma, totalled $\sim 3 \times 10^{11} \text{ g}$. If the Toba eruption released a proportionate amount of H_2SO_4 aerosols, the stratospheric loading after that event could have been $\sim 9 \times 10^{14} \text{ g}$. On the other hand, if the Toba sulphur release was proportional to the output from the 1883 Krakatau eruption, which released a relatively larger percentage of sulphur volatiles, $5 \times 10^{13} \text{ g}$ of H_2SO_4 aerosols from 10 km^3 of dacitic magma⁸, then the stratospheric loading could have been as great as $5 \times 10^{15} \text{ g}$. (Questions regarding rates of aerosol nucleation, saturation, and fallout in a very dense stratospheric aerosol cloud are currently being studied.) Distributed worldwide, these aerosol mass loadings are equivalent to globally averaged peak optical depths of 6 and 33, respectively⁹; regionally, the optical depths could have been even greater. An aerosol optical depth of 6 would have had at least a moderate climatic impact⁹ while an aerosol optical depth of 33 is equivalent to the smoke optical depths used in all but the most severe nuclear winter scenarios^{1,2}.

Basaltic volcanic eruptions may release even greater percentages of sulphur

volatiles^{10,11}. Moreover, observations and recent calculations suggest that large, generally effusive, fissure basaltic eruptions can produce widespread high-altitude aerosols, possibly from convective plumes rising above vigorous fire fountains^{10–13}. A good example of the product of a large basaltic eruption is the massive Roza lava flow (age $\sim 14 \text{ Myr}$) of the Columbia River Basalt Group¹⁴. It is calculated that this single eruption produced more than 700 km^3 of basalt lava (covering an area of $40,000 \text{ km}^2$) in as little as 7 days. The greatest basalt eruption in recent historical times, the Laki (Iceland) fissure eruption¹⁰ of 1783 (volume $\sim 12 \text{ km}^3$) is estimated to have generated about $1 \times 10^{14} \text{ g}$ of H_2SO_4 aerosols⁸. Therefore, the production from the 60 times greater Roza event could have been $\sim 6 \times 10^{15} \text{ g}$, or enough to give a globally averaged aerosol optical depth of about 40. This is equivalent to a smoke optical depth of the size assumed in the most severe nuclear winter models^{1,2}.

Did these eruptions cause sudden and sharp climate cooling? The Toba eruption does coincide with a time of falling global temperatures¹⁵, and there is also some evidence of global environmental changes at the time of the Columbia River Basalt eruptions (mostly between 17 and 14 Myr ago)^{16,17} and at times of other flood basalt extrusions^{11,13}. However, more detailed studies are required to determine whether these coolings were related to the volcanic eruptions, and to assess just how severe the climatic effects of these eruptions really were.

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Basement membrane nomenclature

SIR — In a recent issue of *Nature*, Bluemink *et al.*¹ correctly pointed out the confusion existing over the nomenclature of basement membranes, but their recommendations are not in accord with usage by British, North American, French and Japanese scientists. The International Anatomical Nomenclature Committee² recently recommended the following names for the successive layers of basement membranes: (1) “lamina lucida”, the pale layer in immediate contact with the plasmalemma of the associated epithelial or other cells; (2) “lamina densa”, the dark layer below; and (3) “lamina fibroreticularis”, the incomplete layer (sometimes missing) in continuity with connective tissue. This classification is justified by the fact that the specific components of basement membrane (type IV collagen, laminin and heparan sulphate proteoglycan^{3–5}) are present in the three layers and little or none is found elsewhere. Thus, the three substances are abundant in the lamina densa, forming closely packed cords; they are present in lesser amounts in the lamina lucida as a few loosely arranged cords⁶ and they may occur in the lamina fibroreticularis within “bridges” connecting with other basement membranes (unpublished).

One confusion in the literature is the ambiguity of the term “basal lamina”, which many histologists have used in the past to describe the “lamina densa”, while others consider “basal lamina” synonymous with “basement membrane”. As more is learned of the composition^{3,6,7}, synthesis^{3,7}, structure⁸ and function⁴ of basement membranes, it is important that the terminology be unambiguous. We have adopted the terms defined in the guidelines of the International Anatomical Nomenclature Committee², and urge others to do so.

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Volatile budget of Kilauea volcano

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The volatile content of magma in the reservoirs of active volcanoes has an important bearing on problems of petrogenesis, magma degassing, eruption mechanisms, eruption forecasting and monitoring and the environmental impact of eruptions. Volcanic gas compositions and the S content of glasses are used to infer the volatile content and the degassing systematics of magma from the Kilauea volcano. Our results are combined with data for Kilauea's magma supply and distribution over the past 27 yr to calculate the volatile budget of the volcano.

KILAUEA volcano in Hawaii is detached from the south-east shield of Mauna Loa by the east and south-west rifts (Fig. 1)¹. The roof of the summit magma chamber is ~2 km under the caldera and 1 km under Halemaumau²⁻⁵. Mantle sources at 30–60 km supply parental magma to the chamber⁶⁻⁸. The chamber has been described as a dyke and sill complex with a chamber floor at 6 km, a chamber crown at 1–2 km, and an east-rift duct at 1–2 km between the summit chamber and east-rift magma reservoirs^{1,4,9}. Communication between the summit and south-west rift reservoirs is poorly defined^{10,11}. A relatively steady supply of magma accumulates in the summit chamber before discharge to the surface or down the rifts¹¹. Magma injected into the chamber causes summit inflation, whereas rift discharges cause summit deflation. During summit eruptions, inflation ceases, but deflation may be negligible².

We use the sulphur in glassy east-rift submarine basalts and in glass inclusions to infer the weight per cent of this element in subsurface Kilauea melt. Subtracting the post-eruption residual sulphur in glassy Kilauea fountain spatter from the subsurface concentration, gives the quantity of degassed sulphur (g degassed-S per 100 g melt). Next, we convert volcanic gas analyses, conventionally reported in volume per cent, to mass proportions of S (from SO₂ and H₂S), H₂O, CO₂, Cl (from HCl) and F (from HF). Scaling the mass proportions to the quantity of degassed-S gives the masses of H₂O, CO₂, Cl, and F degassed per 100-g melt. Finally, adding the residual H₂O, CO₂, Cl, and F in glassy fountain spatter gives the weight per cent of each volatile in the subsurface melt. Sulphur is used in our calculations because data for other volatiles in Kilauea glasses are sparse and often highly controversial. We use error propagation equations¹² and the error in the mean ($s/\sqrt{N}$) of the data to calculate the uncertainty in our results.

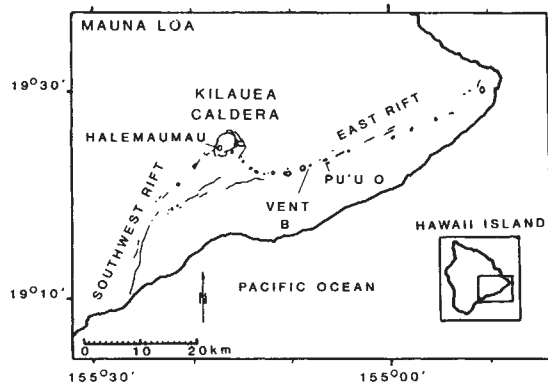


Fig. 1 Sketch map of Kilauea volcano. The current mid-east rift eruption began 2 km south-west of Vent B and extended several kilometres along fissures to the north-east. It subsequently localized at Pu'u O.

Parental volatiles

An active lava lake normally existed in Halemaumau between 1823 and 1924 (ref. 13), reappearing briefly in 1952 and again in the 1967–68 eruption. Lava source vents were active continuously for several months to years during these periods and undoubtedly erupted a significant fraction of parental magma, otherwise retained in the summit chamber (Fig. 2). We postulate that these sustained summit-lava lake eruptions emit a type I volcanic gas derived principally from the parental volatiles in parental magma (Fig. 2a).

The J-series gas analyses of the 1917 and 1918 collections by Jaggard provide the most reliable volcanic gas data from sustained

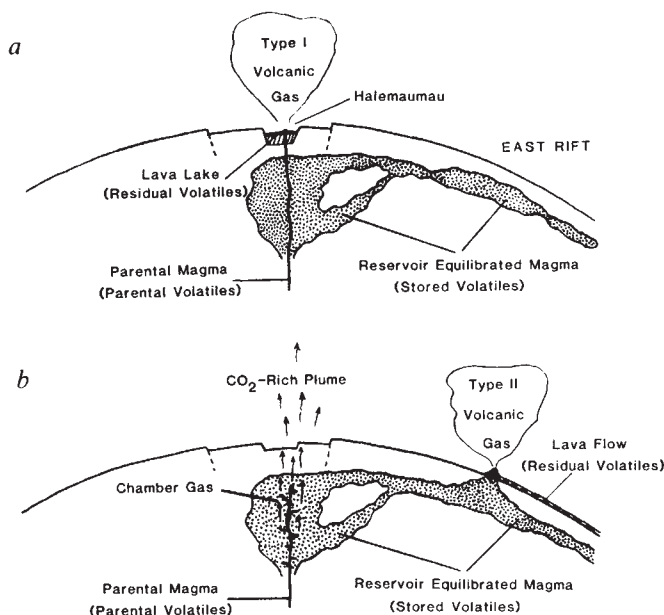


Fig. 2 Schematic diagrams of the magma supply, storage and transport system of Kilauea volcano. The summit chamber is envisioned to be a complex of dykes and sills. The east-rift storage system is assumed to consist of a feeder conduit and dykes. The diagrams are adapted from the models by other investigators¹⁻⁷. *a*, A one-stage degassing process involving a sustained summit eruption of parental magma (solid) emitting type I volcanic gases derived from parental volatiles. *b*, A two-stage degassing process involving chamber gas venting and a rift eruption of reservoir-equilibrated magma (stippled) emitting type II volcanic gases derived from stored volatiles. Chamber gas is released from parental magma as it adjusts to chamber conditions and becomes reservoir-equilibrated magma. The chamber gases that penetrate the hydrothermal system above the summit chamber supply the summit plume. Reservoir-equilibrated magma can also erupt in the summit region.

summit lava lake activity¹⁴. Because his intention was to discover sources of unaltered volcanic gases, Jaggar took samples at a wide variety of lake sites^{15,16}. Although water and oxidation altered most of his samples, several analyses have been restored¹⁴. Table 1 contains the four restored analyses requiring the smallest corrections. Sample J-8 is from a crack in a spatter cone over a 'source of fill' for an active pool¹⁶. This sample is an almost quenched equilibrium composition and needs only trivial corrections within the limits of normal analytical error^{14,17,18}. The three other J-samples are from cones and a grotto at the lake margin¹⁶. They show the effects of fractionation during lava degassing—lower CO₂ and higher H₂O (relative to J-8). Remote infrared spectrophotometry of lava fountain gases during the 1967–68 Halemaumau eruption provides the only other volcanic gas data from a period of sustained summit-lava lake activity; the gases have a composition of 95 vol. % H₂O, 4 vol. % CO₂ and 1 vol. % SO₂ (ref. 19). This analysis is open to interpretation, however, owing to the presence of water vapour in the atmosphere¹⁴. If water is decreased, the composition converges on J-8 (Fig. 3), and we therefore take the composition of J-8 as representative of type I volcanic gases.

We calculate the parental melt H₂O and CO₂ concentrations (Table 2) from J-8 and the residual volatile concentrations in Kilauea fountain spatter²⁰ (Table 2) and the sulphur content of glass inclusions in olivine phenocrysts from a pumice sample of the 1959 summit eruption²¹. We use these inclusions because they have the highest S concentrations reported for Kilauea glasses and show no evidence of sulphide immiscibility. Their size (>0.17 mm) and compositional similarity to associated matrix glasses preclude entrapment of a volatile enriched boundary layer²¹. Insoluble gases, however, may have distilled S from the melt before the inclusions were captured. We therefore use the mean of the highest reported S values, 0.130 ± 0.004 wt %. The lack of reliable data for HCl and HF in the J-series analyses^{14,18} precludes calculation of parental Cl and F concentrations. We therefore infer these quantities by an alternative approach.

The calculated parental H₂O concentration agrees with that measured in the glass inclusions (0.23 ± 0.04 wt %) used to define parental sulphur²¹. Both values are far below those required for H₂O saturation of tholeiitic melt at the chamber depths. The calculated parental CO₂ concentration, however, is much greater than the measured CO₂ content of the glass inclusions (0.08 ± 0.03 wt %)²¹. A depth of ~40 km is required to dissolve the parental CO₂ in tholeiitic melt²², implying that parental melt, with the calculated volatile concentrations, would be greatly oversaturated in CO₂ at the chamber conditions. Most of the parental CO₂, therefore, must enter the chamber as a fluid, which is consistent with observations of CO₂ inclusions in olivine from Hawaiian basalts²³. Hence, at chamber conditions, the parental

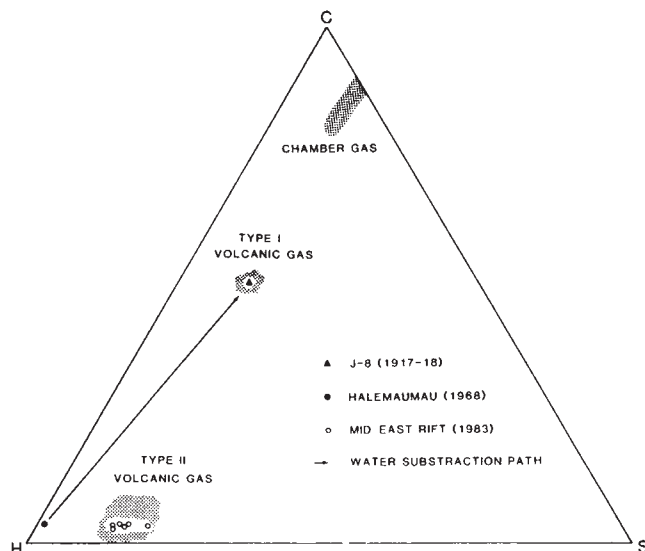


Fig. 3 Compositions (vol. %) of principal Kilauean gases. The components are calculated as follows: C = CO₂ + CO, S = SO₂ + H₂S, and H = H₂O + H₂. Stippled regions estimate composition ranges of the principal gases involved in the degassing of Kilauea magma. The water subtraction path shows the change in composition of the 1968 Halemaumau sample¹⁹ as water is removed. The compositions for J-8 and the 1983 mid-east rift eruption samples are from Table 1.

volatile concentrations in Table 2 are bulk concentrations for the combined parental melt and fluid.

Stored volatiles

We propose that when parental magma remains in the summit chamber, it equilibrates to chamber conditions by separating excess volatiles in a CO₂-rich chamber gas and retaining stored volatiles in reservoir equilibrated magma (Fig. 2b). Reservoir equilibrated magma is either erupted in the summit region or discharged to the rifts¹¹. Rift eruptions, therefore, emit type II volcanic gases derived from the stored volatiles dissolved in reservoir equilibrated melt (Fig. 2b).

The samples for six volcanic gas analyses from the current mid-east rift eruption (Table 1) were collected at 900 °C in January 1983 from Vent B (Fig. 1). They have a low CO₂ content, minimal atmospheric contamination (N₂, O₂ and Ar) and equilibrium compositions (for the magmatic gases) quenched between 950° and 1,030 °C (Table 1, ref. 24). Similar gases continued to be emitted in later phases (T.M.G., unpublished data) when lava became supplied increasingly by direct summit deflation²⁵

Table 1 Vol. % compositions of Kilauea volcanic gases from sustained summit and east rift eruptions

Sample	H ₂ O	CO ₂	SO ₂	H ₂ S	HCl	HF	H ₂	CO	N ₂	Ar	O ₂	T _{eq} (°C)*
Sustained summit†												
J-8	37.09	48.90	11.84	0.06	0.08	ND	0.49	1.51	—	—	—	1,170
J-13	69.29	17.82	10.93	0.11	0.21	ND	1.01	0.62	—	—	—	1,175
J-16	60.42	23.21	14.31	0.21	0.21	ND	0.87	0.74	—	—	—	1,140
J-18	58.14	21.76	17.46	0.30	0.32	ND	1.03	0.93	—	—	—	1,185
East rift‡												
830114-2	81.89	3.83	12.1	0.763	0.172	0.21	1.00	0.070	0.013	0.0002	0.0003	997 ± 6
830115-3	79.40	3.93	13.6	1.72	0.150	0.19	0.94	0.060	0.027	0.0009	0.0011	948 ± 8
830115-4	81.09	3.79	12.5	1.33	0.163	0.17	0.85	0.062	0.017	0.0002	0.0004	952 ± 5
830115-5	82.60	3.56	11.8	0.592	0.151	0.15	0.94	0.065	0.161	0.0021	0.0005	1,003 ± 2
830115-6	80.50	3.53	14.1	0.515	0.174	0.19	0.93	0.079	0.020	0.0031	0.0003	1,032 ± 7
830116-1	76.75	3.38	17.4	1.23	0.170	0.18	0.87	0.059	0.034	0.0005	0.0004	979 ± 1

* ND, No data reported. Equilibrium temperature determined from gas composition without N₂, Ar, O₂.

† After removal of contaminating H₂O, N₂, Ar, O₂ in restoration procedure¹⁴.

‡ Analytical data without removal of atmospheric contamination. All samples were collected in evacuated bottles containing NaOH solution and analysed at Sandia National Laboratories by gravimetric methods, gas chromatograph, ion chromatograph, and infrared absorption.

Table 2 Wt % concentrations of parental, stored and residual volatiles

Volatile class	H ₂ O	CO ₂	S	Cl	F
Parental*	0.30(2)§	0.65(6)	0.130(4)	0.0087(3)	0.0354(20)
Stored†	0.27(2)	0.034(3)	0.070(5)	0.0087(3)	0.0354(20)
Residual‡	0.10(1)	0.015(2)	0.015(1)	0.0080(3)	0.0350(20)

* Wt % concentrations for parental melt oversaturated in CO₂ at chamber conditions, as discussed in text. All concentrations calculated, except for S, which is based on glass inclusion data²¹.

† Wt % concentrations for reservoir-equilibrated melt at 2-km depth. All concentrations calculated, except for S, which is based on data for glassy east-rift submarine basalts²⁶⁻²⁸.

‡ Mean values for CO₂, S, Cl, and F in weak fountain spatter²⁰. H₂O value for fountain spatter from the 1983 mid-east rift eruption (H. R. Westrich, unpublished data).

§ Parentheses enclose propagated error¹² in preceding digit.

|| Based on stored volatile concentrations, as discussed in text.

and outbreaks became localized at Pu'u O vent (Fig. 1). Similar compositions were found again in January 1984 (L. P. Greenland, unpublished data). We assume, therefore, that volcanic gases from the current eruption are representative of the type II volcanic gases (Fig. 3) and derived from stored volatiles in reservoir-equilibrated melt of the summit and east-rift storage systems (Fig. 2b).

We calculate the stored volatile concentrations of reservoir-equilibrated melt from the mean January 1983 volcanic gas composition, residual volatile concentrations²⁰ (Table 2) and the sulphur content reported for glassy rims and crusts of submarine basalts from the east rift (0.070 ± 0.005 wt %). Three investigations, each using different analytical techniques on samples from the same dredge hauls, confirm this value for sulphur content and, moreover, demonstrate that it characterizes east-rift melt extruded from 500 m to at least 5,000 m below sea level²⁶⁻²⁸. We assume this conclusion extends to reservoir-equilibrated melt throughout the summit chamber and the rifts.

The calculated stored volatile concentrations presented in Table 2 are consistent with previous measurements²⁷ on east rift submarine basalt matrix glasses. The deepest (4,680 m) and presumably least degassed sample contained 0.23 ± 0.02 wt % H₂O, 0.025 ± 0.004 wt % CO₂ and 0.071 ± 0.008 wt % S (ref. 27); 0.31–0.35 wt % H₂O has been reported in samples from the same dredge haul²⁹.

The effect of pre-eruption crystallization on the calculated stored volatile concentrations for reservoir-equilibrated melt of the current eruption is probably at the level of uncertainty. Lava temperatures, near vents of the current eruption, range up to 1,145 °C (E. Wolfe, personal communication), suggesting subsurface temperatures of at least 1,155 °C. Heat transfer calculations limit the average pre-eruption cooling of most magma extruded from the east rift to no more than 30 °C (from 1,200 °C)⁹. Temperatures above 1,155 °C correspond to <15% crystallization for Kilauea lava³⁰. Such modest crystallization would have negligible effects on stored volatile concentrations.

A 2-km minimum depth to melt, with the stored CO₂ concentration obtained above (Table 2), is estimated from

$$D = -0.031 + 61.2 (\text{wt } \% \text{ CO}_2) \quad (1)$$

where D is depth in km. Equation (1) includes the pressure

dependence of CO₂ solubility in submarine tholeiitic melt²², assuming a rock density of 2.8 g cm⁻³ (the mean density of Kilauea intrusives and nonvesiculated flows)³¹. A depth of 2.5 km is obtained for a density of 2.3 g cm⁻³, which may better represent flows overlying shallow reservoirs along the east rift³¹. Both values are consistent with depth estimates to the top of the summit chamber, the east-rift duct, and the earthquakes beneath the east rift during the present eruption (R. W. Decker, personal communication).

The stored Cl and F concentrations derived here for reservoir-equilibrated melt of the current eruption are not significantly different from the residual Cl and F concentrations of effervesced Kilauea spatter (Table 2). We infer, therefore, that these values also apply to parental melt and to reservoir-equilibrated melt at all depths in the summit and rift storage system. Nor is there a significant difference between parental and stored water concentrations; this suggests that H₂O concentrations are also almost constant throughout the storage system, implying little or no contamination of reservoir-equilibrated melt by meteoric water during storage. Stored CO₂ concentrations, however, will vary according to equation (1) from 0.10 wt % (6 km) to 0.02 wt % (1 km). The residual CO₂ concentration (0.015 wt %) corresponds to a depth of 0.9 km. Either the shallow degassing of CO₂ is inefficient or the reported CO₂ concentration for Kilauea fountain spatter is too high.

Chamber gas

We calculate the vented chamber gas composition from the differences between parental and stored volatile concentrations. In these calculations, the stored volatile concentrations for H₂O, S, Cl, and F in Table 2 are used for reservoir-equilibrated melt at all depths. The stored CO₂ concentration is estimated from equation (1) as a function of equilibration depth. The chamber gas composition after equilibration at 2-km depth is 10 vol. % H₂O, 79 vol. % CO₂, and 11 vol. % S; allowing for the insignificant difference between parental and stored water content gives 88 vol. % CO₂ and 12 vol. % S. SO₂ would be the predominant S species in the chamber gas (T.M.G., unpublished calculations). Because stored CO₂ is always small compared with parental CO₂, the results are similar for equilibration at other chamber depths (Fig. 3).

Table 3 Kilauea volatile budget, July 1956 to April 1983

Volatile class	H ₂ O			CO ₂			S			Cl			F		
Parental volatiles	18(2)*†	18(2)‡	100§	38(5)*	39(5)‡	100§	7.6(7)*	7.7(7)‡	100§	0.51(5)*	0.52(5)‡	100§	2.1(2)*	2.1(2)‡	100§
Chamber gas	2(3)	2(3)	10	36(5)	37(5)	95	3.5(9)	3.6(9)	46	0.00(7)	0.00(7)	~0	0.0(3)	0.0(3)	~0
Stored volatiles	16(2)	16(2)	90	2.0(2)	2.0(3)	5	4.1(5)	4.2(5)	54	0.51(5)	0.52(5)	100	2.1(2)	2.1(2)	100
Volcanic gas	3.5(7)	3.6(7)	20	0.4(1)	0.4(1)	1	1.1(2)	1.1(2)	15	0.02(2)	0.02(2)	3	0.01(9)	0.01(9)	~0.4
Residual	2.0(3)	2.1(3)	12	0.31(5)	0.31(5)	1	0.31(3)	0.31(3)	4	0.16(2)	0.17(2)	32	0.72(8)	0.73(8)	34.6
Nonerupted	10(1)	10(1)	58	1.3(2)	1.3(2)	3	2.7(3)	2.7(3)	35	0.33(3)	0.34(3)	65	1.3(1)	1.4(1)	65

* Total g × 10⁻¹² calculated as described in text and rounded to significant figures.

† Parentheses enclose propagated error¹² in preceding digit.

‡ (g day⁻¹) × 10⁻⁸ calculated from total g × 10⁻¹² per 9,800 days and rounded to significant figures.

§ Each category (before rounding) as per cent of parental volatile supply.

|| Assumes equilibration of CO₂ in reservoir melt at 2-km depth.

Volatile budget

Table 3 presents the volatile budget of Kilauea volcano for the 322 month period from July 1956 to April 1983. The mean magma supply rate to the summit chamber for this period is $7.2 \times 10^6 \text{ m}^3$ per month (ref. 11). Over the 322 months, this rate gives a total melt accumulation of $5.8 \times 10^{15} \text{ g}$ for magma with a density of 2.8 g cm^{-3} and an olivine content of $10 \pm 5 \text{ wt } \%$ ^{8,32}. From magma accumulation data¹¹, we estimate an uncertainty of $0.5 \times 10^{15} \text{ g}$ in the total melt accumulation. The parental volatile concentrations (Table 2) and the total melt accumulation give the total parental volatile accumulation. During the 322-month period, almost all eruptions involved reservoir-equilibrated magma. Our budget calculations assume, therefore, that chamber gas was degassed from all supplied magma. Subtracting the total chamber gas discharge from the parental volatile accumulations gives the total stored volatile accumulations. Approximately 35% of the magma supplied during the 322 months has erupted; the remaining 65% is still in the rifts¹¹. Using these distribution figures and the residual volatile concentrations (Table 2), we divide the stored volatile accumulations among volcanic gases, residual volatiles, and nonerupted volatiles remaining in the rifts (Table 3). We calculate average g day^{-1} rates by dividing the total accumulation in each budget category by the 9,800 days of the budget period. The reported total accumulations and g day^{-1} rates are rounded to significant figures consistent with the propagated errors for each budget category.

Thus, $18 \times 10^{12} \text{ g H}_2\text{O}$, $38 \times 10^{12} \text{ g CO}_2$, $7.6 \times 10^{12} \text{ g S}$, $0.5 \times 10^{12} \text{ g Cl}$, and $2 \times 10^{12} \text{ g F}$ entered Kilauea's summit chamber over the 322-month period. Degassing, almost exclusively by chamber gas and type II volcanic gases, emitted $5.5 \times 10^{12} \text{ g H}_2\text{O}$, $36.4 \times 10^{12} \text{ g CO}_2$, $4.6 \times 10^{12} \text{ g S}$, $2 \times 10^{10} \text{ g Cl}$, and 10^{10} g F . Non-erupted volatiles (Table 3) stored in rift magma account for most of the undegassed balance. Retention of residual volatiles in erupted lavas also contributes significantly to the undegassed balances of H_2O , Cl , and F .

Discussion

Parental magma carries volatiles into Kilauea's summit chamber at the average g day^{-1} rates given in Table 3. Parental melt transports much of the volatile load in solution, except for CO_2 , which is transported predominantly as a fluid saturating the parental melt from depths of $\sim 40 \text{ km}$. Either the parental magma erupts directly in a sustained summit eruption (Fig. 2a), or it remains in the summit chamber and is transformed to reservoir-equilibrated magma (Fig. 2b).

Sustained summit eruptions of parental magma (Fig. 2a) were commonplace¹³ throughout the century before 1924. These eruptions facilitate a one-stage degassing process. Volatiles effervesce continuously from parental melt approaching the surface, especially in the last 100–50 m as the confining pressure approaches the water vapour pressure of the melt. The effervesced volatiles mix with the CO_2 -rich fluid to produce type I volcanic gases (Fig. 3). The average magma supply rate for the 1956–83 period¹¹ together with parental and residual volatile concentrations (Table 2) give average emission estimates of $12 \times 10^8 \text{ g H}_2\text{O day}^{-1}$, $38 \times 10^8 \text{ g CO}_2 \text{ day}^{-1}$, $7 \times 10^8 \text{ g S day}^{-1}$, $4.2 \times 10^6 \text{ g Cl day}^{-1}$ and $2.4 \times 10^6 \text{ g F day}^{-1}$ for type I volcanic gases during sustained summit eruptions.

Degassing has occurred predominantly by a two-stage process since 1924. The first stage of degassing is the chamber gas venting during the transformation of parental magma to reservoir-equilibrated magma (Fig. 2b). Chamber gas carries off most of the parental CO_2 and about half the parental S (Table 3). The remaining CO_2 and S and almost all the parental H_2O , Cl and F become stored volatiles in the reservoir-equilibrated melt. The equilibrated magma is eventually ejected from the chamber by a summit eruption or (more commonly) by injection into the rifts¹¹. The water content of equilibrated melt (Table 2) corresponds to water vapour pressures of only 10–12 bars (refs 33, 34). As equilibrated melt rises to the surface, therefore, it will effervesce only small amounts of CO_2 , S and H_2O until it reaches

shallow depths of 40–50 m where extensive H_2O degassing can occur. Thus, the second stage of degassing involves type II volcanic gases (Fig. 3) and begins when reservoir-equilibrated magma is almost at the surface during summit or rift eruptions (Fig. 2b).

The two-stage degassing process is expected to be less efficient than the one-stage degassing process. The 1956–83 period characterized by two-stage degassing illustrates this fact. Summing the g day^{-1} rates for chamber gas and volcanic gas (Table 3) gives the following average emission rates: $5.6 \times 10^8 \text{ g H}_2\text{O day}^{-1}$, $37.4 \times 10^8 \text{ g CO}_2 \text{ day}^{-1}$, $4.7 \times 10^8 \text{ g S day}^{-1}$, $2 \times 10^6 \text{ g Cl day}^{-1}$ and $10^6 \text{ g F day}^{-1}$. These rates are higher in one-stage degassing. The two-stage degassing process is less efficient because of the high proportion of H_2O , S , Cl and F in the net accumulation of reservoir-equilibrated magma in the rifts over the 27-yr period¹¹. CO_2 is degassed with about equal efficiency by each process.

The chamber-gas-discharge rate of $37 \times 10^8 \text{ g CO}_2 \text{ day}^{-1}$ calculated for the average magma supply rate over the 27-yr period of the budget (Table 3) agrees with the two CO_2 emission measurements (36×10^8 and $16 \times 10^8 \text{ g CO}_2 \text{ day}^{-1}$)³⁵ available for the summit region. Below and above average magma supply rates¹¹ correspond to chamber gas CO_2 discharges in the range $10\text{--}50 \times 10^8 \text{ g CO}_2 \text{ day}^{-1}$. The magnitude of a possible loss of chamber gas CO_2 by formation of carbonate alteration products in the overlying hydrothermal system³⁶ is not known.

COSPEC-determined SO_2 emissions in the summit plume at various times since 1975 are in the range $1.5\text{--}3.0 \times 10^8 \text{ g day}^{-1}$ (refs 37, 38). The average magma supply rate, however, implies chamber gas discharges of $7.2 \times 10^8 \text{ g SO}_2 \text{ day}^{-1}$ (Table 3). Below and above average magma supply rates imply chamber gas SO_2 discharges of $2.5\text{--}10 \times 10^8 \text{ g SO}_2 \text{ day}^{-1}$. We attribute the comparatively lower measured emissions to the disproportionation of chamber gas SO_2 to $\text{H}_2\text{SO}_4(\text{aq})$ and $\text{H}_2\text{S}(\text{aq})$ in the hydrothermal system and their reaction with basalt lava to form sulphide and sulphate alteration products. These mechanisms are effective in removing SO_2 emanating from a basalt magma chamber and passing through an overlying hydrothermal system at Krafla, Iceland³⁹. Separation of immiscible sulphide melt may also reduce the chamber gas venting of SO_2 .

The summit inflation–deflation record may provide a convenient means for monitoring the future influx, storage and degassing of Kilauea's volatiles. Empirical determinations of summit volume change versus summit tilt, as measured in micro-radians (μrad) by the Uwekahuna tiltmeter, are in good agreement with theoretical predictions and give a mean value¹¹ of $0.33 \times 10^6 \text{ m}^3 \mu\text{rad}^{-1}$. For parental magma with a density of 2.8 g cm^{-3} and containing 10 wt % olivine, this relation implies parental volatile influx coefficients for periods of inflation of $2.5 \times 10^9 \text{ g H}_2\text{O } \mu\text{rad}^{-1}$, $5.4 \times 10^9 \text{ g CO}_2 \mu\text{rad}^{-1}$, $1.1 \times 10^9 \text{ g S } \mu\text{rad}^{-1}$, $7.5 \times 10^7 \text{ g Cl } \mu\text{rad}^{-1}$ and $3.0 \times 10^8 \text{ g F } \mu\text{rad}^{-1}$. Analogous storage coefficients can be derived for stored volatiles injected into the rifts during summit deflation; they are the same magnitude, except for CO_2 ($3.1 \times 10^8 \text{ g } \mu\text{rad}^{-1}$) and S ($6.5 \times 10^8 \text{ g } \mu\text{rad}^{-1}$). Degassing by chamber gas venting is readily calculated from differences in the respective influx and storage coefficients. Volatiles degassed during eruptions can be calculated from the volume of extruded lava, or they can be estimated from the associated deflations and the above coefficients. For example, deflations for extrusions during phases of the present eruption are commonly $15 \mu\text{rad}$ and imply SO_2 emissions of about $2 \times 10^{10} \text{ g}$, which agrees with typical COSPEC emission determinations ($2.2 \times 10^{10} \text{ g}$) (L. P. Greenland, personal communication).

In conclusion, CO_2 and SO_2 'pollution' from Kilauea is, on average, small compared with anthropogenic pollution. For example, the rate of anthropogenic CO_2 pollution ($2 \times 10^{16} \text{ g yr}^{-1}$)⁴⁰ corresponds to $\sim 15,000$ Kilaueas operating on budgets similar to the one in Table 3. Likewise, anthropogenic SO_2 pollution levels ($6.5 \times 10^{13} \text{ g yr}^{-1}$)⁴¹ are equivalent to 380 Kilaueas. Kilauea pollution, however, can be exceptionally intense during large rift eruptions.

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Note added in proof: This average magma supply rate of $7.2 \times 10^6 \text{ m}^3$ per month is thought¹¹ to be a lower limit, making the budget in Table 3 a minimum estimate. The budget neglects degassing of residual volatiles during flow of lava away from source vents.

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Complete nucleotide sequence of the AIDS virus, HTLV-III

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The complete nucleotide sequence of two human T-cell leukaemia type III (HTLV-III) proviral DNAs each have four long open reading frames, the first two corresponding to the gag and pol genes. The fourth open reading frame encodes two functional polypeptides, a large precursor of the major envelope glycoprotein and a smaller protein derived from the 3'-terminus long open reading frame analogous to the long open reading frame (lor) product of HTLV-I and -II.

HUMAN T-cell leukaemia (lymphotropic) viruses HTLV-I, -II and -III, a family of exogenous retroviruses, are associated with T-cell disorders including adult T-cell leukaemia lymphoma (ATLL) and the acquired immune deficiency syndrome (AIDS)¹⁻¹⁴. These viruses share a number of biological and structural features: tropism for OKT4⁺ lymphocytes^{10,15-21}, the ability to produce giant multinucleated cells in culture^{10,17,22-24}, immunological cross-reactivity of some virally encoded proteins^{12,13}, distant nucleic acid sequence similarities^{8,9,25-28} and the preference for magnesium of the viral reverse transcriptase²⁹⁻³¹. Moreover, the genome of HTLV-I and -II as well as the related bovine leukaemia virus (BLV) is somewhat longer than that of other retroviruses^{8,9,25,32-34}. A sequence of 1,600-

1,800 nucleotides is interposed between the 3' end of the *env* gene and the long terminal repeat (LTR) sequences of these viruses^{25,32-37}, the 3' portion of which is a long open reading frame (*lor*)^{32,35,37}. Another feature that distinguishes HTLV-I, -II, -III and BLV from other retroviruses is the marked increase in the rate of transcription initiated within the viral LTR sequences in infected compared with uninfected cells^{38,39}. This phenomenon, *trans*-acting transcriptional regulation, is not observed for other retroviruses⁴⁰ and is probably mediated by the *lor* gene product of these retroviruses³⁸.

Despite the similarity between HTLV-III and the other members of the HTLV-BLV family of viruses, the biology and pathology of HTLV-III differ substantially. Infection with

BH10	GATCTGACCTAGGAATAGGCGACATAGCAAAATAGAGGAGCTGAGACACATCTGTGAGGTGGGACCT	271	381	BH10	CATAATGTTTGGCCACACATGCTGCTGATCCACAGACCCCAACCCACAGAAAGTAACTGGTAAATGTGACA	607	97
BH5	OlySerAspLeuValIleGlnHisArgThrGluAlaGluLeuArgGlnHisAlaGluLeuValIleGluValIle			BH8	HisAsnValTrpAlaThrHisAlaLeuGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	ACCACACAGCAAAAAATCATGAGAGAGCTCCATCTCTTGGATGGGTATGATACCTCCATCTGTAATAAGT	2846	406	BH10	GAATAATTTAACAATGTGAAAAATGACATGTGATGACAGATGATGATGATGATGATGATGATGATGAT	6146	122
BH5	ThrThrProAspLysValHisGlnIleValProMetProMetGlyValGluLeuHisAspAspLysTrp			BH8	GluAsnThrThrSerCysAsnThrSerValIleGlnHisAlaGluLeuValIleGluValIleGluValIle		
BH10	ACATGACAGCTTATGCTGCGCAGAAAAGAGCAGCTGGCTGCTCAATGACATACAGAAAGTATGGGAAATG	2921	431	BH10	CTAAAGCCATGTGTAAATTAACCCCATCTGTGTGTTTAAAGTGCATGATTTAAAGATGATGATTAATACC	6221	147
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	LeuValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	AATGTGGCAAGTCAGATTACCCAGGAGATTAAGTAGAGCAATATATGTAACCTCTTAGAGAGAACCAAGCACTA	2996	456	BH10	AGAGTAAAGTGCAGAAAGATATGATATTTTAAACCTGATATATTAACCAATGATATGATATGATATGAT	6371	197
BH5	AsnProLysSerGlnIleValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle			BH8	ArgGlyValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	ACAAGAGTAAATACCATACAGAGAAGCAGAGCTAGACATGGCAGAAAACAGAGAGATTTAAAGAACCACTA	3071	481	BH10	TATACGTGACAGATGTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	6446	222
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	CATGAGTGTATTATGCCCATCAAAAGATTAATACAGAAATACAGAGCAAGGCAAGGCAAGGCAAGGCAAG	3146	506	BH10	CATTATGTGCT	6521	247
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	HisValCysAlaProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal		
BH10	ATAAGTAAATACAGAGAGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3216	556	BH10	TATGTGTGCT	6596	271
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AsnValSerThrValGlnCysHisMetValIleArgProValIleValSerThrGlnLeuLeuValGlnSerLeu		
BH10	AAATACCATACCAAGAGAACATGGAACATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3371	581	BH10	TCGTGTGATTAATTTTAAAGAACCCACACATACAGAAAGATGATGCTGATCAGAGAGGACAGAGGATA	6671	322
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	SerValIleIleLeuValGlnCysThrArgProAsnAsnThrThrThrThrThrThrThrThrThrThrThrThr		
BH10	GAATGTGTAAATACCCCTCTTGTGTAATATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3446	606	BH10	TCGTGTGATTAATTTTAAAGAACCCACACATACAGAAAGATGATGCTGATCAGAGAGGACAGAGGATA	6746	322
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	SerValIleIleLeuValGlnCysThrArgProAsnAsnThrThrThrThrThrThrThrThrThrThrThrThr		
BH10	TCTATGTAGATGAGGACAGTAAACAGGAGCTAAATAGAGAAACAGATGATTTACTAAACAGAAAGACAA	3521	631	BH10	GCATTGTGATTAATTTTAAAGAACCCACACATACAGAAAGATGATGCTGATCAGAGAGGACAGAGGATA	6821	347
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AlaAlaThr		
BH10	AAAGTGTGCT	3596	656	BH10	ACITTAACACAGATAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	6896	372
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	GAATGATTAATACCATACAGAGAGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3671	681	BH10	GAAGAGGACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	6971	397
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlyValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	TCAGATGTGATTAATCAATTAATGAGCAGTAAATAAAGAGGAAAGGCTGATGCTGCTGCTGCTGCTGCTGCT	3746	706	BH10	TCTGATGATTAATTTTAAAGAACCCACACATACAGAAAGATGATGCTGATCAGAGAGGACAGAGGATA	7046	422
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	AAAGGATGTGAGGAAATGACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3821	731	BH10	CTCCATGACAGATTAACAAATATTAATGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	7121	447
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	ATAGATTAAGGACCAAGTGAACATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3896	756	BH10	GGACAAATAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	7196	472
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	CTGTGATGACAAAGAAATAGTACAGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	3971	781	BH10	GAATGATTAATTTTAAAGAACCCACACATACAGAAAGATGATGCTGATCAGAGAGGACAGAGGATA	7271	497
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlyValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	GACTGATGCTCAGGAAATAGCAGCTAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	4046	806	BH10	ATGACAACTTAGAGGATGACCAACCCACAGAGAGATGATGCTGATCAGAGAGGACAGAGGATA	7346	522
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	GCGATGATGATTAAGAACAGAGATGATTCACGAGAGAACAGGAGAGAGAGATGATTTTCTGTAATTA	4121	831	BH10	GGAGCTTGTCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	7421	547
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlyValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	CCAGAGAGATGGCAGTAAACAAATACATACAGACATGGCAGATTTCCACAGCTGATGATTAAGAGGCC	4196	856	BH10	GGCAGACAAATATGCTGCTGATGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	7496	572
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AlaArgGlnLeuValGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	TGT	4271	881	BH10	TGT	7571	593
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Thr		
BH10	AATAAGAAATTAAGAAATTAATAGACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	4346	906	BH10	CAAGCTCTGT	7646	622
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlnLeuValGlyIleTrpGlyCysSerGlyValLeuLeuValCysThrThrAlaValProTrpAsnAlaSerTrpSer		
BH10	ATATTCATCCACATTTTAAAGAAAGAGGAGGATGTGGGGGTACGTCAGCGGAGAGAAATAGTACATATA	4421	931	BH10	AATAAATCTGTGACAGCAATTTTGGATACATGCTGCTGATGATGATGATGATGATGATGATGATGATGAT	7721	647
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AsnValSerLeuGlnIleTrpAsnAsnMetThrThrThrThrThrThrThrThrThrThrThrThrThrThr		
BH10	GCACAGACATACAAATTAAGAAATAGCAAAACAAATTAACAAATTAACAAATTTTCCGTTTATTAACAGGAC	4496	956	BH10	TTAATACCTCTTAATTAAGAAATGCGCAACCAACAGCAGAGAGAGATGATGATGATGATGATGATGAT	7796	672
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	LeuIleHisSerCysLeuValGlnIleProTrpAsnGlnIleGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	AGCAGAAATACCACTTGGAGAGGACGAGCAAGCTTCTGGAAAGTGTGAGGAGTGTGATGATTAACAGATTA	4571	981	BH10	TGGGCAAGTTGTGGAAATTTTGTATACATACCAATTTGGCTGTGTGATTAATAATTTTATTTGATGATGAT	7871	697
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	TrpLeuSerLeuValGlnIleProTrpAsnIleGlnValIleGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	AGTGACATAAAGATGTGCCAAGAGAGCAAGCAATTCATTAAGGATTAATGAAAGATGATGATGATGATGATGAT	4646	1006	BH10	GCGCTGTGTGTGATTAAGATTTTGTCTGATCTTCTGTGATGATGATGATGATGATGATGATGATGATGAT	7946	722
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlyLeuValGlyIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIleGluValIle		
BH10	TGT	4721	1015	BH10	TCGTTTCAACACCACTCCCAATCCGAGAGGAGCGAGAGGACGAGGAGATGATGATGATGATGATGATGAT	8021	747
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	SerProGlnThrHisAlaSerGlnGlyProGlnArgGlnProGlnArgGlnProGlnArgGlnProGlnArgGln		
BH10	AGCTAAGGGAATGTTTATAGACATCACTAAGAAAGCCCTTCCAAAGATGATCAGAAATACACATCCCACT	4796	70	BH10	QACAGAGACAGATCACTGCAATAGAGGATCTTATGATGATGATGATGATGATGATGATGATGATGATGAT	8096	772
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AspArgAspArgSerIleArgLeuValAlaGlnValIleGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	AGGGATGCTAGATGTTGATTAACACATATTTGGGCTGCTGATCAGAGAGAGAGAGCTGGCATTTGGGTGAGG	4871	95	BH10	TCTGATACCCCTTGGAGAGCTACTCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	8171	797
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	ProAspThrThrIleAspArgGlnLeuValIleValThrArgGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	GATGTCATGAT	4946	120	BH10	GAAGCCCTCAATATTTGGTGAATCTTCTCAAGTATTTGGATGATGATGATGATGATGATGATGATGATGAT	8246	822
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	GlnIleValLeuValTrpTrpAsnLeuValIleGlnValIleGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	GTATTACTTGTGACTTTTTCAGACTGCTGATTAAGAAAGCCCTTATGAGACATAGTATGAGCTAGGTGTGA	5021	145	BH10	AATGCCACACTATAGCATAGCTGGAGGAGCAATAGGATTTAGAAATGATGATGATGATGATGATGATGAT	8321	847
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AsnIleThrAlaIleIleValIleGlnValIleThrArgArgGlnIleValIleValIleValIleValIleValIle		
BH10	ATATCAGAGAGACATACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	5096	170	BH10	CGCCACACTTAGAATATTAAGACAGGCTGGAAGAGATTTTCTGATTAAGATGGGTGGCAAGTGTGCTCAAAAG	8396	863
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	ArgAlaIleProArgGlnIleArgGlnIleGlnValIleGlnValIleGlnValIleGlnValIleGlnValIle		
BH10	AAAGCACCCTTGTGCTGATGTTACGAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	5171	195	BH10	TAGT	8471	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	ATCTCGAAGCTAGAAAACATGAGCAATACATAGATACACACAGACATCAACATGCTGATTTGTCTGCTGCT	8546	
BH10	AGGAGCCACATGAATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	5246	203	BH10	AGAGACACAGAGAGAGAGAGAGGTTTCCAGATCAACCTCAAGCTTCTTAAAGCACAATGACTTACAGAGC	8621	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	ProIle		
BH10	ATTGGCT	5321	531	BH10	ACGTGTGAT	8696	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	TATCTGTGATGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	8771	
BH10	CTGTGACCAATGCTGATGTTGAAAAAGTGTGCTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	5346	546	BH10	CAGATATCCCATGACATTTGGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	8846	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	AGGAGAGAGACCAAGCTTGTACACCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	8921	
BH10	CCGTGCGAGAGAGGCGAGACAGCAGCAGACCTCTCCAGCAGCTGACGATCAAGTGTCTCTGTATCAAA	5621	561	BH10	GAAGTGTGACAGCCCTGACATCTTACATACATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	8996	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	TCGAGCTGCTGACAGAGGATCTTCCGCTGGAGGCTTCCAGAGGAGGCTGCTGCTGCTGCTGCTGCTGCT	9071	
BH10	AGCAGTAAGTGTACATGTAATGACCACTATACCAATAGCAATAGTATGATCACTTTAGTATGATGATCAATA	5696	569	BH10	AGCCCTGAGCTGCTGATATAGAGCTGCTTTTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	9146	
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	Set I		
BH10	TAGT	5771	577	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	AGATGGGACAGCT	5921	47	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuValGluLeuVal			BH8	000AGCTC		
BH10	GGGATGCT	5996	72	BH10	000AGCTC		
BH5	HisGlnIleProLeuValG						

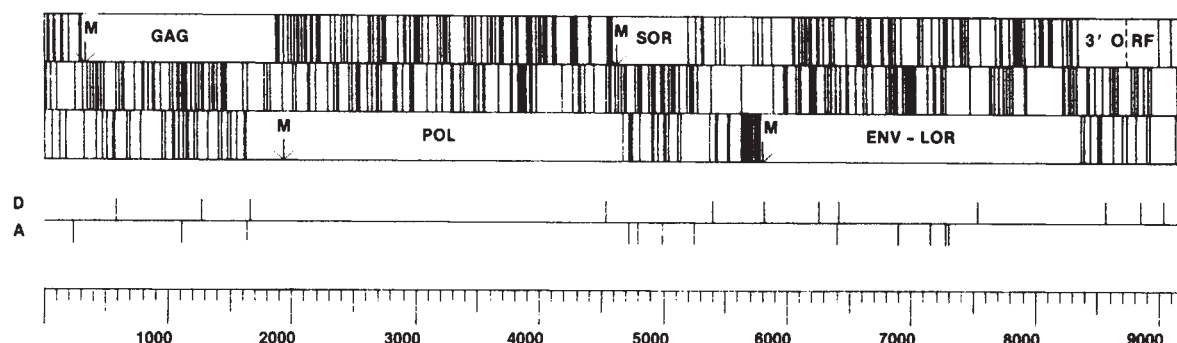


Fig. 2 Distribution of open reading frames in HTLV-III. The positions of termination codons are indicated for each of the three possible reading frames as a vertical line within each box. Thicker lines represent clusters of closely spaced termination codons. The positions of consensus splice donor (D) and acceptor (A) sites⁹³ are shown below. The nucleotide sequence positions are also indicated below, starting from the transcriptional initiation site. The positions of the four largest open reading frames are identified as *gag*, *pol*, *sor* and *env-lor*, and the position of the first ATG codon (M) in each reading frame is indicated. A fifth open reading frame (3'-orf) is found in clone BH8, but not BH10; the broken vertical line indicates the position of a termination codon in BH10.

HTLV-III results often in profound immunosuppression (AIDS), consequent on the depletion of the OKT4⁺ cell population^{10-14,41-43}. This effect is mirrored by a pronounced cytopathic rather than transforming effect of HTLV-III infection upon the OKT4⁺ cells in lymphocyte cultures *in vitro*^{10,11,20}. In contrast, infection with HTLV-I results in a low incidence of T-cell leukaemia lymphoma (an OKT4⁺-cell malignancy)¹⁻⁶. There is evidence also for some degree of immunodeficiency in HTLV-I patients^{6,44}. Infection of primary lymphocytes in culture by HTLV-I and -II results in *in vitro* transformation of predominantly OKT4⁺ cells^{45,46}. A cytopathic effect of HTLV-I infection on lymphocytes is apparent, but the effect is not as pronounced as that in HTLV-III^{16,17,45-48}. HTLV-III differs also from HTLV-I and -II in the extent of infectious virion production *in vivo* and *in vitro*. High titres of cell-free infectious virions can be obtained from AIDS patient semen and saliva and from the supernatant of cultures infected with HTLV-III^{10,49-51}. Very few, if any, cell-free infectious virions can be recovered from ATL patients or from cultures infected with HTLV-I or -II⁵².

To investigate the biological activity of these viruses *in vitro* and *in vivo* and to provide information useful for the development of diagnostic and therapeutic reagents for AIDS, we have determined the complete nucleotide sequence of the HTLV-III provirus.

Genomic structure of HTLV-III

Several closely related clones of HTLV-III DNA were obtained from the H9 cell line infected with HTLV-III present in the blood pooled from several American AIDS patients¹⁰. The complete primary nucleotide sequence of three unintegrated viral clones of 8.9, 5.3 and 3.6 kilobases (kb) in length²⁷ was deter-

mined with the partial sequence from an integrated proviral clone⁵³ (Fig. 1).

The HTLV-III provirus is 9,749 base pairs (bp) long. The overall structure of the provirus resembles that of other retroviruses. The sequences that encode viral proteins are flanked by LTR sequences. The LTR itself is flanked by inverted repeated sequences two nucleotides long (Fig. 1). Four long open reading frames are identified in the viral DNA (Fig. 2).

Long terminal repeat

A detailed analysis of the HTLV-III LTR is presented elsewhere⁵⁴; it is 634 nucleotides long with U3, R and U5 regions of 453, 98 and 83 nucleotides, respectively. The boundaries of these regions of the LTR were defined by localization of the 5'-cap site by S₁ nuclease mapping and by measurement of the length of the strong stop DNA transcript, as well as by determination of the 3'-terminus of the viral RNA by sequence analysis of cDNA clones. A TATAA sequence typical of eukaryotic promoters, as well as the consensus sequence for polyadenylation, are indicated in Fig. 1. A transfer RNA binding site complementary to the 3' end of tRNA^{Lys} is located 3' to the 5' LTR. DNA sequence homologies to the LTR of HTLV-I³², -II⁵⁵ and BLV⁵⁶ are indicated in Fig. 3.

First open reading frame (*gag*)

The structural proteins of the *gag* gene of retroviruses are derived by proteolytic cleavage of a polypeptide precursor encoded at the 5' end of the genome⁵⁷. Such a precursor, 512 amino acids long, could be encoded by the first long open reading frame of the HTLV-III provirus (nucleotides 310-1,869). The definitive assignment of amino-terminus of the *gag* precursor

					PERCENT NUCLEOTIDE IDENTITY TO HTLV-III	
HTLV-III BH10 CLONE U3	8824	GAGAAGTTAGAAGAAGCCAACAAAGGAGAGA	8854		61	
HTLV-III BH8 CLONE U3	8824	GAGAAGTAAGAAGAAGCCAATAAAGGAGAGA	8854			
HTLV-I U3	307	AATAAACTAGCAGGAGTCTATAAAAGCGTGG	337			
HTLV-II U3	268	AATAAAAGATGCCGAGTCTATAAAGGCGCAA	298		51	
HTLV-III U3	9067	TGGCGAGCCCTCAGATCCTGC ATA TAA	9093		62	
BLV U3	152	TGCTGA CCTCA CCTGCTGATAAATTAA	178			
HTLV-III GAG p15	1886	ACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGAT ACAGT ATT AGA AG	1935		76	
HTLV-I BETWEEN GAG & POL	2223	ACTA TCGAAGCTTTACTAGATACAGGAGCAGA CATGACAGTCCTCCGATAG	2274			
HTLV-III BETWEEN SOR & ENV-LOR	5615	CTATCAAGCAGTAAGTAGTACATGTAATG	5644		77	
HTLV-I ENV	5831	CTA CAAAGCACTAATTA TACTTGCAATG	5858			

Fig. 3 Nucleotide sequence homologies between HTLV-I, -II, -III and BLV. Asterisks indicate identity of nucleotides to that present at the same position in HTLV-III (clone BH10 except where indicated otherwise). The nucleotide position of each sequence is shown.

Asterisks indicate amino acids which are identical at the same position as that predicted by the HTLV-III clone BM10 DNA sequence. The positions of the amino acid residues are indicated. Env-1 or sequences L1, L2, and L3 are indicated.

The region between the second and fourth open reading frames (nucleotides 4,674–5,780) also includes an open reading frame (nucleotides 4,588–5,196), capable of encoding a protein 203

amino acids long, which overlaps with the 3' end of the *pol* gene by 86 nucleotides. The 3' portion of this region contains multiple termination codons in all three reading frames and therefore cannot encode a functional polypeptide (Fig. 2). Both the short open reading frame and non-coding region in this part of the HTLV-III genome have been identified by sequence analysis of two independent clonal isolates, BH5 and BH10. In addition, the restriction map of a biologically active HTLV-III proviral DNA clone is indistinguishable from that of the BH10 provirus throughout this region (our unpublished observations with A. Fisher and E. Collalti). For these reasons, we suggest that this unusual sequence is common to replication-competent HTLV-III viruses. We designate the 5' portion of this sequence *sor* (short open reading frame).

The predicted product of the *sor* region would be a polypeptide of M_r 21K. This amino acid sequence shows no significant homology to any known viral or mammalian cellular genes, but there is a region of DNA sequence similarity in the non-coding region (nucleotides 5,615–5,644) with the sequences found in the *env* gene of HTLV-I³² (Fig. 3). Also, the overlap in the open reading frame of the second and third open reading frames is like the structure of the envelope genes of HTLV-I³² and of BLV³³. Thus, it is possible that the *sor* region of HTLV-III and the flanking non-coding region represent a vestigial *env* gene.

Fourth open reading frame (*env-lor*)

The fourth open reading frame (nucleotides 5,781–8,369) could encode a protein 863 amino acids long. There is a short tandem duplication (nucleotides 6,972–6,986 and 6,987–7,001) in the DNA sequence in this open reading frame in one of the two HTLV-III clones (BH10) sequenced (Fig. 1).

The predicted product of the fourth reading frame, *env-lor*, shares many features in common with the envelope gene precursors of other retroviruses, the most striking of which is a hydrophobic region near the middle of the protein (amino acids 519–534)^{32–34,72–75} (Fig. 4). This region of amino acid conservation is preceded in the HTLV-III and other retroviral proteins by an arginine-rich hydrophilic region^{37,76} which includes also the processing site for cleavage of the *env* protein precursors into exterior and transmembrane proteins (see Fig. 5).

The amino-terminal domain of the translation product of the fourth open reading frame also resembles the *env* protein precursors of other retroviruses³⁴. There is a short hydrophobic sequence at the amino-terminus (amino acids 17–37) which may correspond to the signal polypeptide cleaved from the *env* precursor in the maturation process. Moreover, the region of the protein between the putative signal peptide and transmembrane protein is hydrophilic and contains 24 potential asparagine-linked glycosylation sites⁷⁷.

The structure of the amino-terminal domain of the predicted transmembrane region resembles closely that of other retroviruses, with respect to both the relative distribution of hydrophobic and hydrophilic residues and the relative location of cysteine residues^{34,37,73,78–80} (Fig. 5). The HTLV-III *env-lor* protein, however, differs from envelope proteins of other retroviruses in an additional 180 amino acids at the carboxy-terminus^{32,34,37,60,61,73}.

In summary, we believe that the fourth open reading frame encodes an *env* precursor, possibly 826 amino acids long, without the signal peptide sequence. In its mature form it is probably cleaved into a large heavily glycosylated exterior membrane protein about 481 amino acids long and a transmembrane protein 345 amino acids long which may be glycosylated (Figs 1, 5). The size of these predicted products agrees with the detection of a large glycosylated protein of M_r 120–160K in HTLV-III-infected cells which is probably the glycosylated *env* gene precursor⁵⁸ and a smaller, virion-associated gp41 which is probably the transmembrane protein^{12,13}.

Does a *lor* gene exist?

The genomes of HTLV-I, -II and BLV differ from those of the non-acute retroviruses by the presence of a *lor* 3' to the *env*

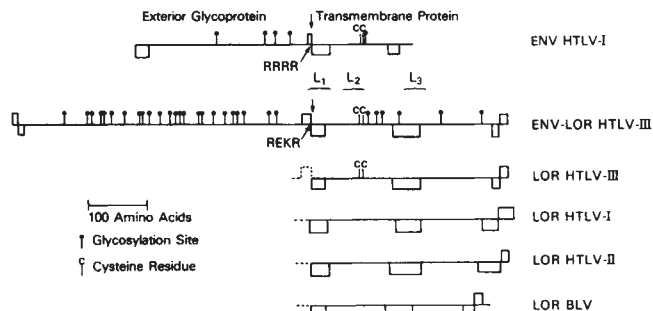


Fig. 5 Structure of *env-lor* product of HTLV-III. The structural features of the predicted *env-lor* product of HTLV-III is compared with those of HTLV-I³², -II³⁵ and the BLV *lor* products^{33,37} and HTLV-I *env* product³². Boxes above and below each line represent hydrophilic and hydrophobic regions, respectively^{94,95}. Also shown are the positions of potential asparagine-linked glycosylation sites⁷⁷ and cysteine residues in the transmembrane proteins. Following asparagine-rich sequences (R, asparagine; E, glutamic acid; K, lysine), the proposed cleavage sites between the exterior glycoprotein and transmembrane protein are shown by an arrow. The scale for amino acid residues is shown at the left. L1, L2 and L3 refer to amino acid sequences which are similar to those of other retroviruses, shown in Fig. 4.

gene^{32,33,35,37,81}. This region of HTLV-I and -II is encoded by a 2-kb mRNA^{81,82} which is translated into M_r 42K and 38K proteins, respectively^{83,84}. There are several reasons to suspect that the 3'-portion of the fourth open reading frame of HTLV-III also encodes a protein with functions similar to those of the *lor* products of the HTLV-I and -II genomes. Most important is the presence of a 2-kb spliced mRNA species in HTLV-III-infected cells including the 3', but not the 5' portion, of the fourth open reading frame (our unpublished observations with S. K. Arya and G. Chan). This mRNA is long enough to include ~1,000 nucleotides of the 3' portion of the fourth open reading frame.

The overall structure of the *lor* product thought to be synthesized from the carboxy-terminal 1,000 nucleotides of the fourth open reading frame of HTLV-III is similar in structure to that predicted by the nucleic acid sequence of the *lor* regions of HTLV-I, -II and BLV^{32,35,37}. This similarity includes the size of the *lor* polypeptide, the presence of three hydrophobic regions spaced approximately the same distance apart and the presence of a carboxy-terminal hydrophilic region (Fig. 5). Such a protein may mediate the observed *trans*-activation of transcription of the viral LTR in HTLV-III-infected cells³⁹, as suggested already for the *lor* product of HTLV-I, -II and BLV^{38,85}.

There is a sequence of 292 nucleotides between the end of the fourth open reading frame and the beginning of the LTR. The purine-rich sequence preceding the LTR is similar to that of the site of second-strand DNA initiation⁸⁶ (Fig. 1).

We propose that the fourth open reading frame encodes two functional polypeptides, one that serves as the precursor for the major envelope glycoprotein and a second derived from the 3' end of the open reading frame, corresponding to the *lor* gene of other HTLV-BLV viruses.

Heterogeneity in HTLV-III viruses

Sequences from different clones of HTLV-III allow an analysis of the level of sequence diversity of the virus. A comparison of clones BH8 and BH5 with BH10 (Fig. 1) demonstrates a 0.9% base pair polymorphism in the coding regions of the genome and a 1.8% base pair polymorphism in the non-coding regions. In addition, three 1–3-bp deletions are noted in the non-coding regions, as well as a 15-bp sequence present in one copy in clone BH8, but repeated tandemly in the *env-lor* region of clone BH10. In the coding regions, most nucleotide differences are in the third codon positions; of those that predict amino acid differences between clones, most are non-conservative substitutions. The resultant differences in viral protein function remain

to be determined. Of note is the presence of a fifth open reading frame (nucleotides 8,344–8,991), designated 3' *orf*, present in clone BH8 but truncated in BH10 (Fig. 2).

The heterogeneity among HTLV-III clones shown here could represent sequence divergence developing in culture in a given individual over a period of time, or polymorphic differences in viruses from different individuals. Diversity among different HTLV-III isolates seems to be greater than that between different HTLV-I isolates^{6,53}. Different isolates of HTLV-III comprise a spectrum of closely to distantly related viruses. Heterogeneity of proviruses within a given individual, however, has not been noted. Furthermore, a cloned HTLV-III provirus from another individual differs in 18 of 31 restriction enzyme sites mapped compared with clone BH10; the restriction enzyme map of this isolate did not change over a period of several months in culture (our unpublished observations with B. Hahn and G. Shaw). Thus, it is likely that most of the divergence among the HTLV-III clones analysed here represents differences in strains in different individuals. The full extent of this diversity in the HTLV-III family of viruses, however, remains to be determined, and is probably necessary in estimating possible differences in antigenicity of viral proteins. These differences could be important factors in the design of agents useful for viral detection or for therapeutic agents.

Discussion

HTLV-III demonstrates structural and functional similarities to other members of the HTLV-BLV family of retroviruses. Like HTLV-I³² and BLV³³, the *gag* precursor of HTLV-III lacks a small phosphoprotein situated between the amino-terminal *gag* protein and the major capsid protein. Significant amino acid homologies exist between the major capsid and the basic *gag* proteins of HTLV-III and the corresponding proteins of HTLV-I and BLV. These homologies could account for the immunological cross-reactivity among these proteins^{12,13}. Similarities of nucleic acid sequence in several areas of the genome are consistent also with previous hybridization data^{26,27}. The presence of conserved amino acid sequences in the transmembrane region of the major envelope glycoprotein also might account for the cross-reactivity in the envelope gene products of HTLV-III and HTLV-I, -II and BLV^{12,13,87,88}. The possibility that the 3' end of the fourth open reading frame may encode a protein similar to the *lor* product of HTLV-I, -II and BLV^{33,35,37,81,83,84} might explain the *trans*-acting transcriptional activation common to these viruses^{38,39,85}. Alteration of the cellular transcriptional apparatus by an HTLV-III *lor* product may be responsible, at least in part, for the specific cytotoxic effect of HTLV-III on OKT4⁺ cells.

Previous studies also have highlighted differences between HTLV-III and other retroviruses. Although detectable, nucleic acid homology between the genomes of HTLV-III and other members of the HTLV-BLV family of viruses is low, reflected in differences in primary nucleotide sequence. The structure of the envelope glycoprotein of HTLV-III is different also from that of HTLV-I, -II and BLV, consistent with recent reports of differences in the size of the major envelope glycoproteins⁵⁸ as well as the differences in the type of cell receptors recognized by these viruses (refs 89, 90 and our unpublished observations with M. Popovic). Another unusual feature of the HTLV-III genome is the presence of a short open reading frame, *sor*, between the *pol* and *env* genes. The location of the *sor* region suggests that it may be evolutionarily related to the *env* gene. Although the function of this region is unknown, it too may account for some of the unusual cytotoxic and immunological properties of the HTLV-III virus.

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Isolation of a homoeo box-containing gene from the *engrailed* region of *Drosophila* and the spatial distribution of its transcripts

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The engrailed locus of Drosophila melanogaster has the characteristics of both a homoeotic gene and a segmentation gene; like a homoeotic gene, it specifies the development of specific compartments of the Drosophila embryo (the posterior compartments of each segment), and, like mutations of segmentation genes, lethal alleles of engrailed affect also the pattern of segmentation of the embryo. Here we report that like many of the homoeotic genes of the bithorax and Antennapedia complexes, engrailed has a 'homoeo box' sequence; also, like the segmentation gene fushi tarazu, the engrailed gene displays a periodic pattern of expression in Drosophila embryos.

THE molecular events responsible for the generation of a complex organized pattern of differentiated tissues during embryogenesis are poorly understood. Genetic studies of pattern formation in *Drosophila* have, however, led to the discovery of many genes which seem to be involved in the regulation of these processes. The recent cloning of some of these developmental regulatory genes means that their molecular activities can now be investigated.

A common strategy in the embryogenesis of insects is the subdivision of cell groups into segmental units which develop according to different programmes¹. In *Drosophila*, the initial determination of the segmentation pattern occurs during the cellularization of the syncytial blastoderm²⁻⁵. The proper subdivision of the embryo into segments requires the correct expression of at least 15 genetic loci thought to be differentially activated in response to a maternally-established morphogen gradient⁶. Mutations in these genes cause severe defects in the segmentation pattern and are therefore embryonically lethal. Further regional specification imposed on the segmentation pattern is necessary to ensure the correct assembly of the various structures into a fully-functional fly. This process is thought to involve a series of successive subdivisions of the developing organism into compartments (units of cell lineage) which are differentially determined⁷. The first evidence for these developmental compartments was obtained from clonal analysis on imaginal disks⁷, the compartments representing the domains of activation for the homoeotic genes^{4,7,8}. Bithorax complex (BX-C)⁹ and *engrailed* genes^{8,10} provide much experimental evidence to support this hypothesis. The *engrailed* locus is required for maintenance of the border subdividing the wing blade into anterior and posterior compartments⁸. Clones of cells homozygous for the *engrailed*¹ (*en*¹) allele generated in the wing disk of *en*¹/+ larvae by mitotic crossing-over induced by X rays, developed anterior-like characteristics and did not respect the antero-posterior demarcation line. Therefore, *engrailed* (*en*)

may be considered a homoeotic gene involved in the specification of the posterior compartments.

Normal expression of this gene is involved also in subdividing the embryonic segments into anterior and posterior compartments¹⁰; it has the phenotype of a segmentation gene⁶. Various segmental fusion patterns have been discovered in the cuticular phenotypes of embryos of different lethal alleles of the *engrailed* locus¹⁰, possibly because the *engrailed* gene encodes several separate functional units taking part in the different steps of the segmentation process¹⁰. Finally, as this gene seems to exhibit both homoeotic and segmentation properties, it may contain genetic components characteristic of both homoeotic and segmentation genes.

Several homoeotic genes in the *Antennapedia* complex and the bithorax complex have a common element, the homoeo box¹¹. This conserved sequence appears in multiple copies in many metazoan genomes¹² and the corresponding genes have been isolated using homoeo box-containing DNA fragments as probes in genomic library screening^{11,14-16}.

We describe here the isolation of a gene from the *engrailed* region using the homoeo box homology. On the basis of the temporal and spatial distribution of the transcripts from this homoeotic homologous sequence, we conclude that it is the *engrailed* gene.

Isolation of clones

Homoeotic genes of *Drosophila* have been isolated using short DNA fragments containing *Antennapedia* (*Antp*) and *fushi tarazu* (*ftz*) homoeo box sequences as probes for screening genomic libraries under conditions of reduced hybridization stringency¹¹. When *Drosophila* genomic Southern blots are hybridized under the same reduced stringency conditions with a probe containing the *Ultrabithorax* (*Ubx*) homoeo box, however, some bands of hybridization appear which cannot be detected in corresponding experiments with *Antp* and *ftz* homoeo box sequences (W.J.McG., unpublished results). To isolate the clones corresponding to these sequences, a genomic library was screened with a small, 450-base pair (bp) *Ubx* gene

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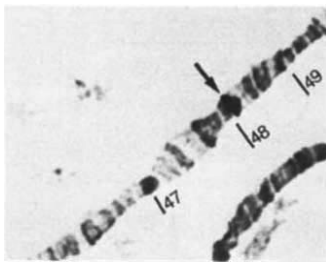


Fig. 1 *In situ* hybridization of biotinylated λ F116 DNA to salivary gland chromosomes. The hybridized probe was detected by an immunoperoxidase method²⁹ and the chromosomes stained with Giesma and photographed. The section divisions of chromosome 2 in the region of interest are indicated and the site of hybridization marked by an arrow.

probe carrying the homoeo box. Approximately 30,000 plaques (three genome equivalents) from the Charon-4 *Drosophila* library of Maniatis *et al.*¹⁷ were screened, and clones which map to six different cytological positions on polytene chromosomes obtained. Three overlapping phage inserts from λ F21, λ F70 and λ F116 were mapped cytologically to position 48A on the right arm of chromosome 2 by *in situ* hybridization (Fig. 1); as this corresponds to the cytogenetic location of the homoeotic gene *engrailed*¹⁰, these clones were chosen for further studies.

The location of homoeo box homology in the clones was determined by hybridizing Southern blots of restricted DNA with a *Ubx* homoeo box probe under conditions of reduced stringency. With all three clones, the probe hybridized to a 0.92-kilobase (kb) *Eco*RI fragment (see Fig. 2) corresponding to the indicated region in the restriction endonuclease map of λ F21 in Fig. 3. A subclone, pF7036, constructed in the vector pAT153, was used to further define the site of cross-homology by Southern blot analysis. A 613-bp *Eco*RI/*Sal*I fragment in the left part of pF7036 contains sequences cross-hybridizing with the *Ubx* homoeo box.

The 48A homoeo box homology was characterized further by probing whole genome Southern blots in conditions of reduced hybridization stringency. The number of bands detected with the 48A probe (Fig. 2, lane 3) is much lower than in the corresponding experiment with a *Ubx* homoeo box-containing probe (lane 4). Although the 48A homoeo box is detected with *Ubx* probe, no positive signal is observed for the *Ubx* homoeo box when the whole genome Southern blot is probed with the 48A probe. This is probably because of less efficient transfer of the 7.5-kb *Ubx* homoeo box-containing fragment.

Additional evidence that the three λ clones from the 48A region correspond to parts of the *engrailed* locus was obtained by hybridizing pF7036 to a dot blot of λ clones (provided by Dr T. Kornberg) from a chromosomal walk in which a series of *engrailed* mutations have been mapped¹⁸. After hybridization at high stringency, positive signals were seen for three overlapping clones from the chromosomal walk (data not shown). No other positive signals were detected when the same blot was probed with *Antp* and *Ubx* homoeo box DNA in conditions of reduced hybridization stringency. Therefore, the chromosomal walk which was tested does not appear to contain other regions having homoeo box homology.

The 48A homoeo box was used also as a probe to isolate cDNA clones from a cDNA pool prepared from early embryos (1.5–5 h) by Michel Goldschmidt-Clermont. Several identical cDNA clones of 0.5 kb were isolated (see Fig. 3) from the screen, indicating that the 48A homoeo box is transcribed at this early stage of development.

DNA sequences

The fine structure of the 48A region containing *Ubx* homoeo box homology was analysed further by DNA sequencing of homologous fragments from genomic and cDNA clones. A

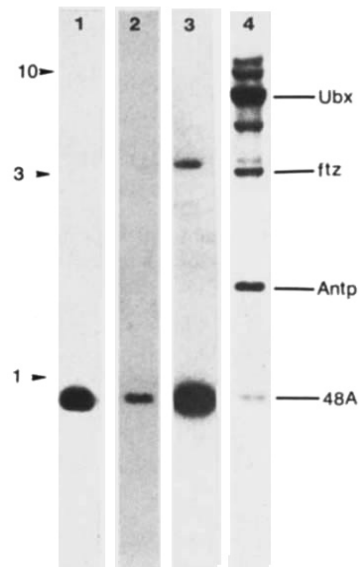


Fig. 2 Homoeo box homologies analysed by Southern blot experiments. Cloned and genomic total DNAs were digested with *Eco*RI and the resulting Southern blots probed with homoeo box-containing DNA fragments in conditions of normal and reduced hybridization stringency. Lane 1, λ F70 probed with *Ubx* at reduced stringency; lanes 2 and 3, *Drosophila melanogaster* genomic DNA probed with the 613-bp *Eco*RI/*Sal*I fragment from pF7036 in conditions of normal and reduced hybridization stringency, respectively; lane 4, *D. melanogaster* genomic DNA probed with *Ubx* at reduced stringency. The migration distances of 10-, 3- and 1-kb size standards are indicated, together with the locations of homoeo box-containing fragments from *Antp*, *ftz* and *Ubx*.

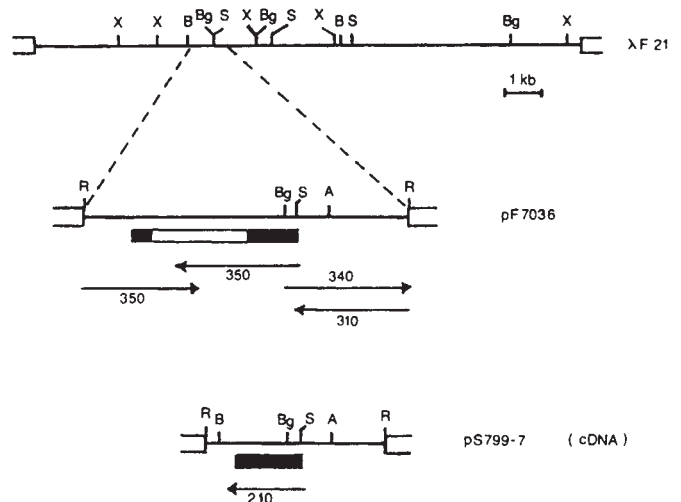


Fig. 3 Restriction maps of clones originating from 48A. Black boxes identify the regions containing homoeo box homology. Restriction enzymes: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; S, *Sal*I; X, *Xho*I. Arrows below the map represent sequence determination by the primer extension method³⁰.

genomic segment of 921 bp and a cDNA region of 210 bp were sequenced (Fig. 3) and we found that the organization in a 600-bp genomic region containing the homoeo box (see Fig. 4) displays several unexpected features. First, the homoeo box is split into two parts by an intron of 280 bp. Comparison of the cDNA with the genomic DNA sequence indicates that they are non-contiguous between positions 84 and 365, sites which fulfill also the sequence criteria for splice junctions¹⁹. Similarly, an intron occurs in the 3' region of the yeast *al* mating-type gene which shares homology with the homoeo box²⁰. We cannot exclude the possibility, however, that transcripts containing

Fig. 4 Organization of DNA sequences with homoeo box homology. Genomic DNA sequence (5' to 3') of a 600-bp region including the two exons (boxed) of the 48A homoeo box. The sequence on the other strand also containing relatively strong homoeo box homology, the 48A anti-box, is underlined. Sequences upstream of the 48A anti-box are shown from the point where there is a theoretical start of a 'homoeo box' on this strand. The known splice sites are indicated by arrowheads.

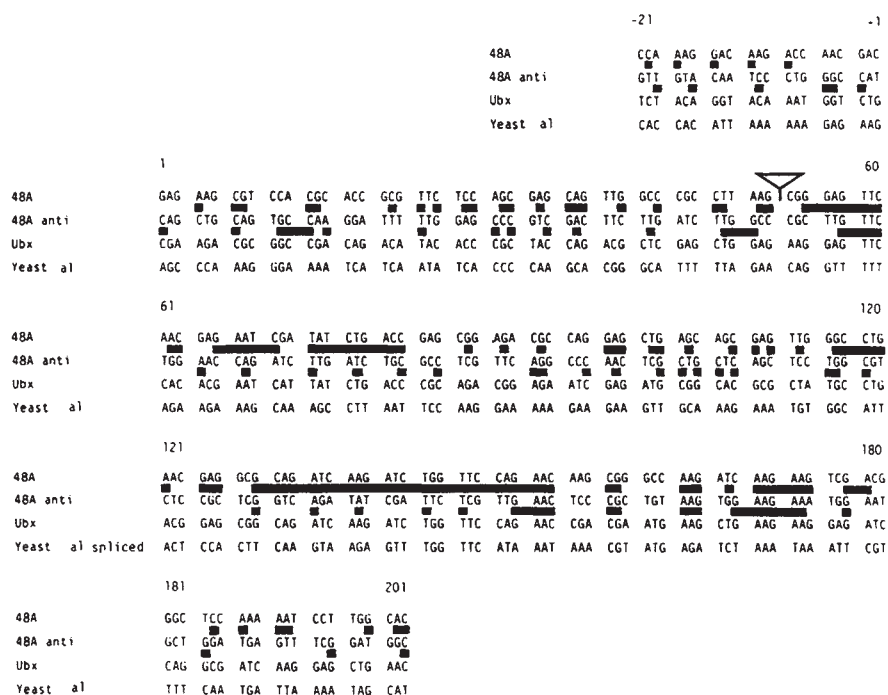
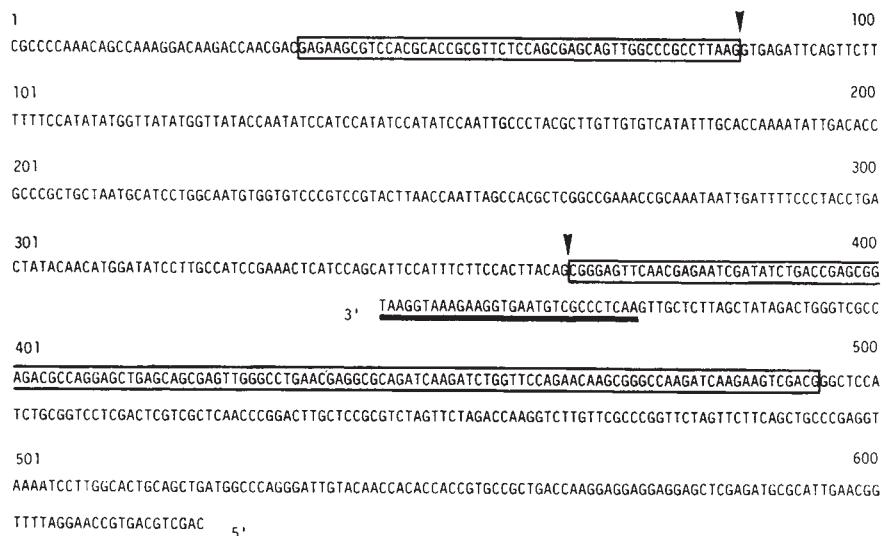


Fig. 5 DNA sequence of the 48A 'boxes'. DNA sequences (5'-3') from pF7036 are aligned with the homologous region from *Ubx* and the yeast *al* mating-type gene. The sequence is shown from -21 (21 bp upstream of the homology) to +201 (21 bp downstream of the homology). Codons of the common open reading frame are aligned (frame 1 begins with nucleotide 1). Black bars underline the nucleotide homology between *Ubx* and the 48A 'boxes'. The position of the intron is indicated by a triangle.

other versions of the 48A homoeo box may exist as results of inefficient splicing or initiation of transcription at an alternative site. It is of interest to note the existence of some cross-homology (38%) between the 5' region of the *Ubx* homoeo box and the 48A intron sequence just upstream of the acceptor splice site (position 365-314).

The 48A homoeo box is more diverged relative to *Antp*, *ftz* and *Ubx* than any other homoeo box characterized so far. At the nucleotide level (Fig. 5), the cross-homology with *Ubx* and *Antp* is only 58 and 53% respectively, considerably lower than the 75–79% found for other *Drosophila* homoeo boxes¹². Only in the case of an interspecies comparison between mouse and *Ubx* homoeo boxes have cross-homologies of <70% been observed¹⁴. The level of cross-homology between the 48A homoeo box and other *Drosophila* homoeo boxes, however, is not distributed uniformly throughout the 180-bp sequence. Thus, in the 3' region there is a stretch of 60 bp 72% homologous to the corresponding part of *Ubx*, in strong contrast to the 52% homology found in the other 120 bp of the 48A homoeo box. Such a localized distribution of the cross-homology may be crucial for detection of cross-hybridization to the *Ubx* homoeo box under the conditions of reduced hybridization stringency.

used normally¹¹. When the same 60-bp 3' region of 48A is compared with *Antp*, the degree of cross-homology is less striking (65%). Within this region there is also a higher ratio of GC homology with respect to *Ubx* (35%) than *Antp* (29%). These differences are probably responsible for the inability to use the *Antp* homoeo box as a probe for detection of the 48A homoeo box on whole genome Southern blots under our reduced hybridization stringency conditions. Therefore, if other highly diverged homoeo boxes exist in the *Drosophila* genome which do not contain subregions of 'high' cross-homology with respect to any of the different homoeo boxes used as probes, they have probably escaped detection.

Another provocative feature of the 48A homoeo box region is the 30-bp sequence on the opposite strand (underlined in Fig. 4) which is 53% homologous with the 3' end of the *Ubx* homoeo box (Fig. 5); seven of 10 residues are conserved relative to the *Antp* homoeo domain (Fig. 6). Furthermore, this 30-bp region, the 48A anti-box, is part of an open reading frame which overlaps most of the 48A homoeo box (see Figs 4, 5); the conceptual translation of this region yields an amino acid sequence that shares some properties with the *Ubx* homoeo domain (Fig. 6).

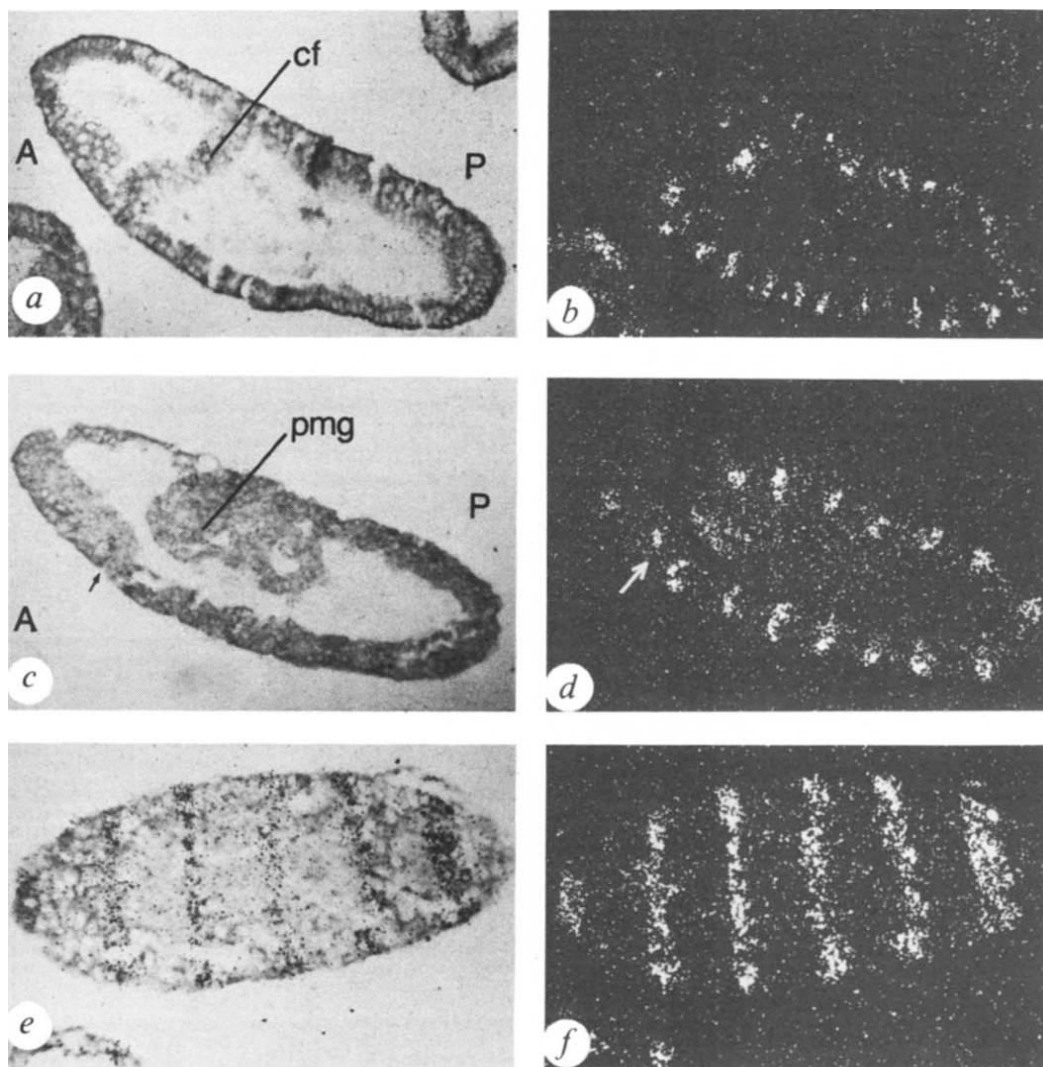


Fig. 8 Localization of transcripts homologous to pF7036 in embryonic tissue sections. Embryonic tissue sections were prepared and hybridized to ^3H -labelled probes as described by Hafen *et al.*²². Both bright-field (a, c and e) and dark-field (b, d and f) photomicrographs of 3-week autoradiographic exposures are shown. a, Longitudinal section of an embryo 3½-h old. b, The embryo photographed with dark-field optics. c, Longitudinal section of an embryo at the 6-h stage. The position of the ventral invagination of the cephalic furrow is indicated by an arrow. d, The same 6-h embryo shown with dark-field illumination. An arrow indicates the position of the cephalic furrow. e, Tangential section of an embryo of about the same stage as c. f, Photomicrograph of the same tangential section with dark-field optics. A, anterior; P, posterior; cf, cephalic furrow; pmg, posterior midgut invagination.

box-containing genomic fragment of the subclone pF7036 was analysed by Northern blotting. Two low-abundance poly(A)⁺-containing RNA species were detected in the three embryonic stages investigated (Fig. 7). A major transcript estimated to be 2.6 kb long (by comparison with single-stranded DNA and RNA standards) seems to be present throughout embryogenesis. However, the weaker signal intensity in the lane representing 0–6-h embryos suggests that only the older embryos of this group transcribe this RNA at a significant level. These embryos (after 4 h of development) are undergoing germ-band extension and visible segmentation.

After this initiation of transcript accumulation, a peak concentration is observed during the 6–12-h stage which is then succeeded (12–24 h) by a strong reduction in the relative abundance of the 2.6-kb transcript.

A 1.3-kb 48A homoeo box-containing transcript is present also during embryogenesis, but seems to be significantly less abundant. Using a single-stranded probe, we have not been able to detect any RNA transcribed from the 48A anti-box-containing strand. Therefore, both of the transcripts detected with the double-stranded probes probably contain 48A homoeo box sequences.

Localization of transcripts

A direct approach to solving the problem of gene identification is to investigate how the transcripts are distributed spatially during development. Recently, several homoeotic genes have been analysed successfully by *in situ* hybridization to tissue

sections^{5,11,22–24}. In these cases the transcripts were restricted spatially to regions where the functions of the respective genes are probably required for proper development. To test whether the 48A homoeo box is included in transcripts displaying temporal and spatial accumulation patterns expected from a gene with an *engrailed*-like function, pF7036 DNA was used as a hybridization probe on tissue sections.

Successive developmental stages of wild-type embryos were investigated and a clear periodic pattern of hybridization is first seen in early gastrulating embryos (3–4 h). Figure 8 shows such an embryo in which the cephalic furrow has formed and the process of germ-band extension is being initiated. Along the ventral side, a series of at least 11 narrow hybridization bands (each of only 1–2 cells in width) is visible (Fig. 8b). In addition, two groups of cells in the cephalic furrow are relatively strongly labelled. The hybridization signals, which also can be seen dorsally, seem to be spatially restricted to the region of the embryo posterior to the cephalic furrow. This posterior localization of silver grains can first be distinguished above the background level when the furrow starts to invaginate (data not shown). Coincident with the extension of the germ band, the accumulation of transcripts is increased and can be seen most clearly at the time of termination. At this stage of embryogenesis (~6 h after fertilization), the mesodermal and ectodermal cell layers lying along the ventral side of the embryo have extended posteriorly, causing the invaginated posterior midgut to move anteriorly along the dorsal surface^{25,26}. Evenly spaced bands of cells labelled with silver grains, separated by wider sections of unlabelled cells, appear along the ventral and dorsal side of the

embryo (Fig. 8d). Two clusters of hybridization signals are seen anterior to the ventral invagination of the cephalic furrow (Fig. 8c, d) and posteriorly 14 bands of hybridization are repeated along the extended germ band. The domain of hybridizing cells just posterior to the cephalic furrow seems to correspond to the position of the mandibular segment²⁷. Accordingly, the 14 hybridization bands represent labelled parts of the mandibular, maxillary and labial segments of the head, three thoracic and eight abdominal segments. The two clusters of hybridization signals anterior to the ventral invagination may represent the anterior-most head segments (clypeolabrum and hypopharynx). Furthermore, the clusters of silver grains are, on average, only 50% of the width of the unlabelled regions. The anterior region of the posterior midgut invagination also shows hybridization signals slightly above background.

A tangential section of an embryo at the same stage (Fig. 8e, f) shows the parallel arrangement of hybridization bands on the surface of the embryo. Regions of unlabelled cells were clearly wider than the stripes of silver grains and discrete bands of hybridization signals were detected also in embryos of older stages.

Conclusions

We describe here the isolation and characterization of a homoeo box-containing gene which maps outside the previously identified clusters of homoeo box sequences in the *Antennapedia* and *BX-C* complexes (ref. 11; W.J.McG., unpublished data). Sequence analysis of the 48A homoeo box homologous sequences of genomic and cDNA clones reveals additional features distinguishing this gene from other previously characterized homoeotic loci. The 48A homoeo box seems to be split into two parts by a short intron; the degree of homology with other homoeo boxes is relatively low both at the DNA and protein level, amounting to less than 60%.

Using the 48A homoeo box as a probe in whole genome Southern blotting experiments in conditions of reduced stringency, only one or two additional DNA fragments can be detected, indicating that the 48A box belongs to a rather small subgroup of homoeo box-containing genes. Other potentially spliced versions of the 48A homoeo box in which the more strongly diverged 5' end is substituted by another weakly homologous homoeo box sequence, may exist at some stage of the *Drosophila* life cycle. Another intriguing possibility arises from the conceptual translation of an open reading frame on the opposite strand to the 48A homoeo box region. A short sequence of 10 amino acid residues in this open reading frame has a similar pattern of homology with respect to the rightmost part of the *Ubx* homoeo domain as observed for the 48A homoeo domain. We have no evidence at present for transcription of these sequences. The fact that the more highly diverged 5' sequence of the 48A homoeo domain is encoded in a different exon may indicate the existence of a separate domain. Accordingly, the homoeo domain may be a composite of two subdomains

with independent functions. It is also interesting that two different transcripts of 2.6 and 1.3 kb contain sequences which originate in part from this region; this could result from an alternative use of promoter and/or splicing signals. The occurrence of premature termination or a specific degradation intermediate, however, cannot be excluded.

The temporal and spatial distribution of transcripts from the homeotic homologous sequences of the 48A genomic clone pF7036 is largely consistent with previous genetic information on the *engrailed* gene.

Based on data from Northern and *in situ* hybridization analyses, the 2.6-kb transcript containing the 48A homoeo box first starts to accumulate at the time when gastrulation is initiated (3.5 h after fertilization), coinciding with the stage at which the subdivision into posterior and anterior compartments may occur^{1,10}. Furthermore, the increase in transcript accumulation seems to parallel the formation of visible segments in the last-phase of germ-band extension, reflecting the importance of *en*⁺ expression for the proper formation of segments. The *ftz* gene is expressed in domains of the wild-type blastoderm which seem to coincide with the parts deleted in homozygous *ftz* embryos⁵ and correspond in their width to segments as determined by laser ablation experiments. In *en* the pattern of hybridization does not correspond precisely to the segmental deletions and fusions observed in the homozygous embryos of several lethal *en* alleles, as these mutants show a much more variable pattern of defects^{10,28}. However, the observed spatial distribution of 48A homoeo box-containing transcripts displays a feature expected from a gene expressed in the posterior compartments: only about one-third of the cells seem to be localized in the labelled region and this correlates well with the sizes estimated for posterior compartments at early developmental stages^{7,10}. Thus, the hybridization pattern could reflect a segmental arrangement of labelled posterior compartments separated by unlabelled cells of the anterior compartments.

A limitation in our ability to interpret the observed distribution pattern, however, is imposed by the fact that two different transcripts are detected by the probe. As a consequence, a differential spatial distribution for the two transcripts would not be detected. Further *in situ* hybridization analyses to obtain a more complete understanding of the functional complexity of the *engrailed* gene are in progress.

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An X-ray corona in SS Cygni?

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A recent model¹ for the X-ray emission of non-magnetic cataclysmic variables suggests that most of the observed hard X-ray flux originates in a radiatively-cooling quasi-hydrostatic corona surrounding the white dwarf as it accretes from its companion. We present here some results from a ~ 7.5 -h EXOSAT observation of the dwarf nova SS Cygni in its quiescent state, which confirm many of the predictions of this model. In particular, they show that the X-ray luminosity, L_X , and bremsstrahlung temperature, T , obey the predicted relationship $L_X \propto T^{3/2+2\epsilon}$. The results further suggest that the emitting region covers an appreciable fraction $f \sim 0.1$ of the white dwarf's surface, and also that transport processes into the white dwarf cause most of the luminosity to emerge at soft X-ray/extreme ultraviolet wavelengths.

The EXOSAT² observations of SS Cygni were made on 1984 December 4. SS Cygni was detected in both the medium energy (ME) experiment³ (~ 2 –20 keV; 'hard' X rays) and the Channel Multiplier Array (CMA)⁴ (~ 0.04 –2 keV; 'soft' X rays) at flux levels similar to those measured previously in quiescence (ref. 5, and refs cited therein). Figure 1 shows part of the ME and CMA X-ray light curves. Flaring, on time scales from tens to hundreds of seconds, dominates the behaviour seen in both 'hard' and 'soft' X-ray bands, with overall variability being factors of ~ 5 and ~ 10 , respectively. From ref. 1, the predicted (bremsstrahlung) time scale is

$$t_{\text{rad}} \approx 30 \rho_{-10}^{-1} T_8^{1/2} \text{ s} \quad (1)$$

where ρ_{-10} is the coronal density in units of $10^{-10} \text{ g cm}^{-3}$ and

T_8 is the temperature in units of 10^8 K . For consistency with the model, we require $\rho_{-10} \sim T_8 \sim 1$ (see Fig. 2 of ref. 1), which is in good agreement with the observed flaring time scales. The coronal gas is hydrostatic, and cools by bremsstrahlung, so the instantaneous X-ray luminosity is $L_X \propto A f H_r \rho^2 T^{1/2}$, where A is the area of the white dwarf; f , the fraction covered by coronal gas and $H_r \sim 10^8 T_8 M_1^{-1} R_9^2 \text{ cm}$, the radial scale height of the corona, with M_1 and R_9 being the mass and radius of the white dwarf in units of $M_\odot$ and 10^9 cm , respectively. Because ρ will decrease slowly as the gas cools, we expect $L_X \propto T^{3/2+2\epsilon}$ with ϵ small and positive. The derivation of this result given in ref. 1 neglected velocity gradients in the quasi-hydrostatic gas, which is not in general justified. However, a full hydrodynamical calculation (M. D. Smith and A.R.K., in preparation) confirms the result.

To check this prediction, we have examined the way in which a temperature-sensitive 'hardness ratio' of the observed ME counts varies with the total count rate. The observed variation for SS Cygni is shown in Fig. 2, where we plot the mean 'hardness ratio' for the data divided into 15 count-rate bins of equal width. It is clear from Fig. 2 that the mean 'hardness ratio' (that is, spectral temperature) increases systematically with count rate (that is, incident flux). This agrees well with data from Ariel 6 (A. Bennetts and M. J. Ricketts, unpublished data). The actual form of this correlation is as expected, as can be seen from the comparison between the data and the curves of the form $L_X \propto T^n$ folded through the ME detector response, which are also shown in Fig. 2. A good match to the observed data is achieved for $n = 2$, although the range $1.5 < n < 2.2$ is acceptable. We stress that we have not carried out extensive tests on more complex functional fits, and that this parameterization should not be regarded as a true fit to the data. Adopting the $n = 2$ curve, the required normalization implies

$$L_X \approx 2 \times 10^{32} T_8^2 D_{100}^2 \text{ erg s}^{-1} \quad (2)$$

where D_{100} is the distance to SS Cygni in units of 100 pc. This leads to

$$f \approx 2.8 M_1 R_9^4 \rho_{-10}^{-2} T_8^{1/2} D_{100}^2 \quad (3)$$

for the surface fraction covered by coronal gas. Given the uncertainties in the parameters in equation (3), this suggests an appropriate range of $f \sim 0.1$.

A further prediction of the coronal model is a strong correlation between soft and hard X-ray emission. First, when a hard X-ray flare begins, the soft X rays should also peak because conduction into the white dwarf is followed by rapid re-emission from its surface as soft X rays ($T \sim 10^5 \text{ K}$). Second, as the coronal gas itself cools, it will become opaque with respect to its own radiation (the equivalent hydrogen column density N_H is of the order 10^{23} cm^{-2}). This occurs when the metals can hold on to their K-shell electrons, at $T \sim 10^7 \text{ K}$ (the ionization balance, as in the solar corona, is between collisional ionization and radiative recombination). Thus, after a few cooling times following the start of a hard X-ray flux, there should be a second peak in the soft X-ray emission. If individual flares are seen, a time delay of a few cooling times may be expected between peak hard and soft X-ray emissions. If, on the other hand, the flares overlap, a broad correlation, centred on zero lag, but of width of a few cooling times, will result. Figure 3 shows the cross-correlation between the hard (ME) and soft (CMA) X rays observed with EXOSAT. There is a good correlation (coefficient ~ 0.25) at zero lag, with a half-width of $\sim 100 \text{ s}$, which is in good agreement with what is expected for overlapping flares with the cooling time, t_{rad} , given by equation (1). (Note that an earlier version (preprint) of this paper claimed a soft X-ray lag of $\sim 60 \text{ s}$, as would occur if individual flares were seen; this lag is spurious, however, arising from a computational error in a cross-correlation routine. Note also that because the energy bands of the ME and CMA have very little overlap, these

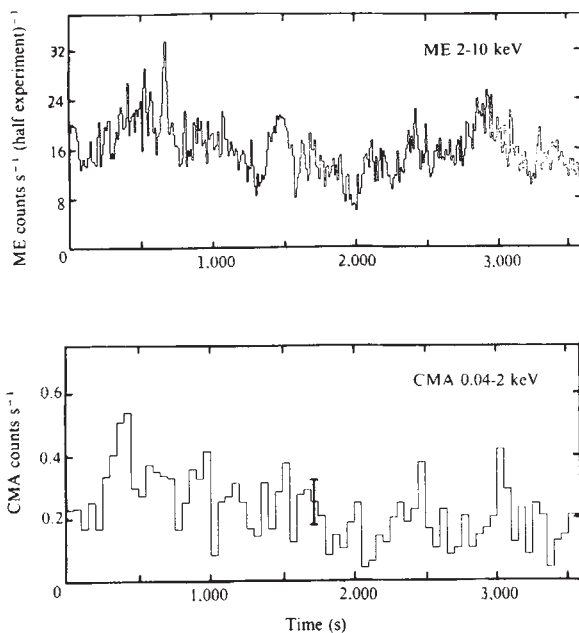


Fig. 1 Hard (~ 2 –10 keV) and soft (~ 0.04 –2 keV) X-ray light curves of SS Cygni obtained with the EXOSAT ME and CMA (+ thin Lexan filter) detectors. The time resolution for the ME light curve is 10 s and for the CMA light curve, 50 s. Only part of the observation is shown here. Time zero on this plot is 1983 December 4, 04:03 UT.

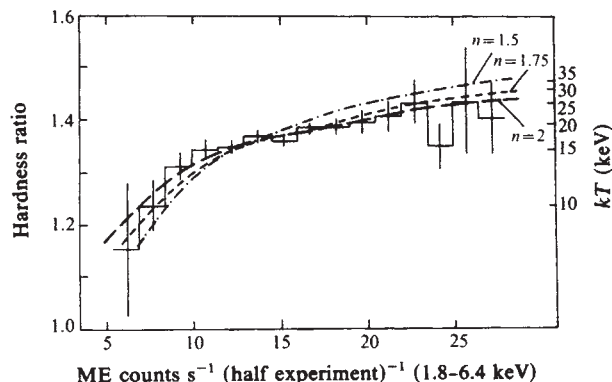


Fig. 2 Mean 'hardness ratio' = $((3.3-6.4)\text{-keV flux}/(1.8-3.3)\text{-keV flux})$ for the ME observations of SS Cygni plotted as a function of $\sim 1.8-6.4\text{-keV}$ count rate. The data are divided into 15 count-rate bins of equal width. The equivalent bremsstrahlung temperatures are shown on the right-hand axis. The dashed curves correspond to relationships of the form $L_X \propto T^n$ folded through the response of the ME detectors for $n = 1.5, 1.75, 2.0$. These curves are normalized to fit the data, using a least-squares technique. Error bars shown are $\pm 1\sigma$.

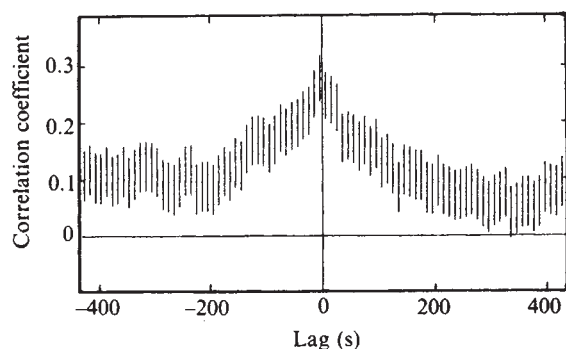


Fig. 3 Cross-correlation between the hard (ME) and soft (CMA) light curves with a time resolution of 10 s. Data from the entire EXOSAT observation have been used. Maximum correlation occurs for soft X rays with a lag of $+60 \pm 15$ s. The error bars indicate the 95% confidence region.

instruments are dominated by the hard (>2 keV) and soft (<1 keV) X-ray components, respectively. The contribution of the hard ($T_8 > 1$) component to the CMA count rate is $<30\%$.

The accretion luminosity L_{acc} of SS Cygni in quiescence is uncertain, but is unlikely to be less than $\sim 10^{33} \text{ erg s}^{-1}$. From equation (2) we see that only a small fraction of L_{acc} ($<10\%$) emerges as hard X rays, although half of L_{acc} is potentially available from the (unstable) boundary-layer gas¹. This supports the suggestion¹ that most of the energy is removed from the corona by conduction into the white dwarf and is subsequently re-emitted as soft X rays. As even weak magnetic fields have a decisive effect in channelling transport processes, it is possible that transient surface magnetic fields generated by dynamo action in the white dwarf envelope may cause the quasi-coherent, soft X-ray pulsations seen in dwarf novae at outburst^{6,7}. This idea is developed in the accompanying paper⁸.

The present results give strong support to the coronal X-ray emission model developed in ref. 1: the temperatures, luminosities and inferred densities all appear to be in accordance with the expectations of this model, whereas the estimate given in equation (3) suggests that a large fraction of the white dwarf surface is involved.

The correlation between hard and soft X rays and the $L_X \propto T^{3/2+2\epsilon}$ behaviour is very striking; in particular, it is difficult to see how the latter can be produced other than in a corona. Our ability to examine the detailed behaviour of SS Cygni simultaneously in the hard and soft X-ray bands demonstrates the

value of the long, uninterrupted observations which are possible with EXOSAT, and the advantages of the soft X-ray band-pass of the CMA detectors in the study of the soft X-ray/extreme ultraviolet emission in cataclysmic variables. It will be important to try to extend these results to other systems, although this will not be easy as they are all considerably fainter than SS Cygni. However, much can be done with SS Cygni itself: in particular it will be interesting to see whether long-term changes in the normalization of the L_X-T relationship occur. These would indicate a change in the covering fraction, f . It will also be important to try to correlate X-ray and optical variability and to attempt, using EXOSAT, to follow SS Cygni through an outburst. Such a study is planned for the near future.

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The origin of soft X-ray pulsations in dwarf novae at outburst and the DQ Herculis phenomenon

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A recent study of X-ray emission from non-magnetic cataclysmic variables¹ concludes that most of the hard (≥ 2 keV) flux is produced in a corona formed by the accreting material around the white dwarf. EXOSAT observations of SS Cygni (see accompanying paper)² strongly support this model, confirming several of its detailed predictions. The model suggests that soft (≤ 2 keV) X-ray production is controlled largely by transport processes into the white-dwarf body which remove most of the energy from the accreting matter. I point out here that, because even weak magnetic fields can exert a marked influence on transport processes, there is a natural explanation for the quasi-periodic oscillations seen in soft X-ray observations of dwarf novae at outburst^{3–5}. I suggest further that the puzzling behaviour of the 'DQ Herculis' system (DQ Her, V533 Her and AE Aqr) may be explained in this light.

Dwarf-nova soft X-ray pulsations were first detected by the HEAO-1 satellite during an outburst of SS Cygni³; subsequently they were detected again from SS Cygni and also from U Gem⁴. The character of these pulsations is complex, but can be described as that of an oscillator having an almost stable period, P , but suffering random changes in phase at irregular intervals. In the two observations of SS Cygni, the periods P were 8.8 and ~ 11 s, and in U Gem ~ 27 s, and the coherence times (measured in cycles) were, respectively, 28, 2 and 1 (see ref. 4 for a full discussion). Unlike optical pulsations from dwarf novae, which come in many forms⁶ but do not involve much of the total luminosity, the large amplitude ($\geq 15\%$) of the soft X-ray pulsations signifies that the accretion luminosity is modulated. This, and the fact that X rays are hard to produce elsewhere, show that the site of the pulsation phenomenon must be deep in the gravitational potential well, that is, very close to the white-dwarf surface. The large luminosities involved create almost insuperable difficulties in trying to adapt models for optical pulsations involving non-radial oscillations of the white dwarf (see ref. 6 for a review).

The alternative class of model, in which the white dwarf is assumed to have a permanent magnetic field strong enough to channel the accretion and thus cause modulation at the period of the white dwarf's spin, P_{spin} , also suffers from serious defects. About 20 magnetic cataclysmic variables of this type (AM Her systems and intermediate polars) are known (none of them are dwarf novae); they have several easily understood properties which contradict those observed in dwarf-nova pulsations. In particular, they all exhibit coherent pulsing at P_{spin} , in both hard and soft X-ray emissions, at all times. In dwarf novae, soft X-ray pulsations appear only at outburst, are only quasi-coherent, have variable periods and are never accompanied by hard X-ray pulsation^{7,8}.

In fact, it is the absence of an accompanying hard X-ray pulsation which offers a clear hint as to the possible origin of the dwarf-nova pulsations. The coronal model for X-ray production from non-magnetic cataclysmic variables¹ emphasizes the importance of transport processes, especially electron thermal conduction, in carrying energy into the white-dwarf body from which it is radiated as soft X rays. The absence of coherent pulsing in hard X rays rules out the presence of a strong, permanent white-dwarf magnetic field of strength B with $p_{\text{mag}} = B^2/8\pi$ greater than the gas pressure p_{cor} in the corona. But even extremely weak magnetic fields can totally control the directionality of transport processes; we require only that the Larmor radius of the electron should be smaller than its mean free path. If this weak magnetic field is 'permanent' (that is, anchored in the degenerate core of the white dwarf) coherent pulsing at P_{spin} will be seen in soft X rays but not in hard, as found in the recent observation of VW Hyi during superoutburst⁵, and, as I shall suggest, may be occurring in the DQ Her systems. If, on the other hand, the weak magnetic fields are 'transient' and generated by dynamo action in the white dwarf envelope, quasi-periodic quasi-coherent soft X-ray pulsations will naturally result, again without any accompanying hard X-ray pulsation.

I examine first the case of transient dynamo-generated fields which should be produced most readily during outbursts of dwarf novae, when differential rotation in the white-dwarf envelope is greater because of the greater accretion of high angular-momentum material near its equator. Indeed, the changes in period, in particular the tendency for P to shorten as the luminosity rises, are easy to understand if P represents the rate of rotation of the outer envelope, and is determined by competition between accretion of angular momentum and other (for example, viscous) torques linking the envelope to the core⁸. Unfortunately, dynamo theory cannot yet make firm quantitative predictions but some immediate consequences are useful. I follow here the simple discussion given by Meyer and Meyer-Hofmeister⁹ in the context of accretion disk theory.

A small poloidal field B_θ is wound up by differential rotation to produce a toroidal field B_ϕ at a rate

$$\frac{dB_\phi}{dt} = 2\pi r B_\theta \frac{d\Omega}{dz} \quad (1)$$

Here (r, ϕ, z) are cylindrical coordinates and $\Omega(z)$ is the angular velocity of the surface layers. This process operates over a time t_w , during which the flux tube expels gas from its interior, becomes buoyant and rises about the scale height H_r . Ultimately, turbulent magnetic diffusion reconnects the field lines and re-creates a small poloidal component

$$B_\theta = \gamma B_\phi \quad (2)$$

where γ (assumed to be $\ll 1$) is a parameter of the theory. From equations (1) and (2) we find

$$t_w \sim \frac{1}{2\pi\Omega(\gamma R/z)} = \frac{P}{4\pi^2(\gamma R/z)} \quad (3)$$

where $z = \Omega/|d\Omega/dz|$, and I have set $r \sim R$. As t_w gives the lifetime of a magnetic loop, the quantity

$$C = \frac{z}{4\pi^2\gamma R} \quad (4)$$

measures the coherence, in cycles P , of the loop structure and hence of any soft X-ray pulsation; the next loop will appear somewhere else on the white-dwarf surface. (I assume that new flux is generated, as in solar active regions, in restricted parts of the white-dwarf surface, giving only a few new 'spots' superimposed on a general 'filigree' background—compare maps of the solar surface fields in the Ca^+ chromospheric emission network.) After evaluating the buoyant velocity v_b of the rising flux tubes, and setting $t_w = H/v_b$, we have, using equations (1) and (2)

$$p_{\text{mag}} \sim \frac{B^2}{8\pi} \sim 4 \times 10^6 T_5 \left(\frac{P}{10\text{s}}\right)^2 \left(\frac{C}{3}\right)^{-2} \text{ dyn cm}^{-2} \quad (5)$$

for the magnetic pressure in a loop, where T_5 is the white-dwarf surface temperature in units of 10^5 K . This is to be compared with gas pressures $p_{\text{cor}} \sim 10^6 \text{ dyn cm}^{-2}$ and $p_{\text{BL}} \sim 4 \times 10^5 M_{16} \text{ dyn cm}^{-2}$ in the corona and boundary layer, respectively^{1,10}, where $10^{16} M_{16} \text{ g s}^{-1}$ is the accretion rate. If $p_{\text{mag}} \ll p_{\text{cor}}$, p_{BL} , the loops will be crushed to the white-dwarf surface and reconnect there, giving a filigree network and no large-amplitude soft X-ray pulsation. For the loops to influence transport processes, they must penetrate the accreting gas, that is, we require $p_{\text{mag}} \geq 10^6 \text{ dyn cm}^{-2}$. (Clearly the loops can never become strong enough to channel the accretion flow dynamically, that is, such that $p_{\text{mag}} \gg p_{\text{cor}}$, p_{BL} , as they are anchored in material of only comparable inertia.) From equation (5) we see that this is only possible for strong loops of low coherence, that is, for $C \sim 3$; from equation (4), it appears this is most likely to occur at outburst, when differential rotation is strongest and thus z is smallest.

The soft X-ray oscillation observed over 10 days during a superoutburst of VW Hyi appears to be coherent, with a 14-s period⁵. Here the magnetic field must be fixed in the degenerate core of the white dwarf: loops due to field-winding would always tend to erupt at fixed positions on the white-dwarf surface, giving a coherent pulsation at the spin period, which must therefore be 14 s, implying a very rapidly rotating white dwarf of mass $\geq 0.7 M_\odot$.

Besides VW Hyi, only two other cataclysmic variables show coherent (optical) pulsing which does not appear at hard X-ray energies: namely, DQ Her and V533 Her. AE Aqr shows rather weak and fairly soft coherently-pulsed X rays¹¹. The periods, P , of all three, 71, 63 and 33 s respectively, are much shorter than any other coherently-pulsing cataclysmics. As noted by Warner¹², these three 'DQ Her' systems form a subgroup distinct from the AM Her systems and intermediate polars mentioned above. The absence of hard X-ray emission in DQ Her and V533 Her, and its weak presence in AE Aqr, are strikingly analogous to the observations of VW Hyi. I suggest that these DQ Her systems have quite weak permanent magnetic fields, amplified by dynamo action as in VW Hyi in a superoutburst. The reprocessing of pulsed soft X rays in the surrounding accretion disk accounts for their optical properties; it may be possible to understand the abrupt cessation of the pulsation in V533 Her¹³ in this way. Detection of pulsed soft X-ray emission from DQ Her or V533 Her would support this suggestion, but may be frustrated by interstellar absorption.

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A model for diagenesis in proto-planetary bodies

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In the earliest stage of planetary-body formation, a proto-planet presumably consists of loosely-accumulated solid grains, or clusters of grains, with ice mantles and water-ice grains. Physical and chemical reactions, similar to diagenesis¹ in the terrestrial permafrost², may occur and may be enhanced by an interfacial water layer between solid grains and water-ice at temperatures below the melting point of water-ice. The thickness of the interfacial water layer increases with rising temperature and/or pressure in the proto-planetary body. The model I propose here eases the thermal constraints imposed by earlier models^{3,4} of low-temperature aqueous alteration in primitive extraterrestrial materials in which formation of an aqueous medium promoting these alterations required complete melting of the water-ice. In the proto-planetary body, low-temperature alterations, for example, the formation of hydrated silicates, will take place because of the presence of an interfacial water layer.

In the cooling solar nebula, solid particles may be formed by condensation from a vapour in the inner part of the nebula⁵⁻⁷ and it has been suggested^{5,6} that the terrestrial planets and asteroids themselves are formed by accumulation of these solid particles at temperatures ~ 450 K. Transport of dust particles from a hot to a cooler region of the solar nebula may produce fluffy aggregates⁸, which, if transported to a low-temperature region (< 210 K), would initiate condensation of water-ice and/or clathrates⁶, that is, conditions favourable for the gradual accumulation of solid particles (dust) or clusters of particles with ice mantles or included ice grains.

Mechanisms for the accumulation of small grains into larger clusters, aggregates, small planetary bodies and all intermediate stages of growth, on cosmologically-reasonable time scales, have been proposed^{9,10}, but for the purpose of this discussion I shall define two stages in the evolution of a planetary body: a proto-planetary and a sub-planetary stage. A proto-planetary body may form by rapid accumulation of solid grains because of gravitational instabilities over a period of at least 10^3 yr. This proto-planet (a few km in size) consists essentially of primordial matter, including solar nebula condensates and interstellar grains, recently accumulated outside the turbulent regions of the solar nebula¹⁰; it will be a loose agglomeration of grains, mainly silicates, and clusters of grains with variable thicknesses of ice coatings. In the proto-planet, random mixing of dust and icy-dust grains will give rise to localized regions with considerable diversity in the dust/ice ratio. The second, sub-planetary stage may evolve from the proto-planetary stage on a time scale of $\sim 10^4$ yr⁹; the sub-planet (500–1,000 km in size) will consist primarily of reworked solar nebula material³, for example, layer silicates may form by low-temperature (< 400 K) aqueous alteration of primordial olivine and pyroxene¹¹.

In previous models of planetary-body, asteroid and comet evolution, an internal heat source has been proposed to explain the observed geochemical and mineralogical data^{3,4}. These models discuss the formation of liquid water above the melting point of water-ice, which may exist³ in asteroids as a transient 'internal atmosphere' sealed-in from the low-pressure environment by a layer of ice at the surface with a lifetime determined by the sublimation rate of the water-ice^{3,12}. Indeed, in a fluffy body of minimum diameter 1.2 km, the decay of short-lived ^{26}Al , present in unusually high abundance as inferred from CV chondrites, will raise the internal temperature to 1,800 K¹³. Furthermore, both the ^{26}Al half-life (7.2×10^5 yr) and the sub-planetary-body accretion time (10^4 yr) are short, hence it has been argued⁴ that their overlap would be fortuitous.

My proposed model for dust evolution in a proto-planetary body draws on studies of diagenesis in low-temperature terrestrial environments such as the permanently-frozen surface soils in Antarctica. In general, diagenesis requires the presence of water¹. Studies of soils from Wright Valley (Antarctica) show that substantial chemical weathering occurs at temperatures between 233 and 268 K¹⁴⁻¹⁶. However, equilibrium mineral assemblages will not occur in these soils as chemical reactions at these low temperatures are kinetically controlled^{17,18}. Chemical weathering in the Antarctic environment is enhanced by the presence of (unfrozen) liquid-water at the interfaces of solid grains (for example, silicates) and water-ice². (Weathering of silicates usually involves the transformations of mica into vermiculite and vermiculite into montmorillonite^{14,15} and the precipitation of carbonates, sulphates, chlorides, limonite (that is, poorly crystalline hydrated oxides of iron) and zeolites¹⁶.) The liquid-like character of interfacial water disappears below 193 K (ref. 19); in addition, its presence is favourable for comminuting the silicate grains whose mechanical disintegration further enhances chemical reactions by exposing new surfaces²⁰.

An interfacial water layer in permafrost has the following typical characteristics²: (1) a zone of enhanced structural order is surrounded by less-ordered water at the interfaces of solid grains and ice; (2) its density is less than the density of free water; (3) its viscosity and specific volume are greater than those for free water; (4) an increase of overburden, or total pressure, at constant temperature increases the amount of interfacial water; (5) its thickness depends on the ambient temperature and on the nature of the solid grain. For example, with decreasing temperature, its thickness decreases exponentially from 10 to 80 Å at 273 K to a minimum of ~ 6 Å at 193 K. In general, the thickest interfacial water layers have been observed on clay-ice mixtures.

In the permanently frozen soils of Antarctica, diagenetic mineral formation occurs on a time scale of 10^4 – 10^6 yr^{14,15,21} and the amount of time necessary for accumulation of micron-sized dust into larger clusters and aggregates¹⁰ and the evolution of proto-planetary bodies into sub-planetary bodies⁹ may be $\sim 10^7$ yr. Thus, the time scale for diagenetic alterations in proto-planetary bodies is sufficiently great provided the dust resides in a thermal regime in which an interfacial water layer will persist.

That interfacial water layers can exist at temperatures below the melting point of water-ice in the dust-ice mixture of a proto-planetary body eases the constraints of earlier models requiring the melting of all ice. In the proto-planetary body the temperature increase may be provided by an ambient background level of ^{26}Al , perhaps produced in our Galaxy at a constant high rate by common nova explosions²². Indeed, I have calculated that a diffuse supply of galactic ^{26}Al , $\sim 15\%$ of the amount inferred from CV chondrites¹³, is sufficient to raise the internal temperature to at least 193 K. As the proto-planet develops physically, the total pressure will increase with the increase in volume and, with increasing pressure and temperature, interfacial water layers between silicate grains and ice will increase in thickness. Thus, low-temperature diagenetic reactions similar to those observed in terrestrial permafrost may occur in a loosely-packed mixture of silicate grains and ice in a proto-planetary body with the result that some of its primordial matter loses its primitive character and that new minerals will form *in situ*. The driving force for these reactions is similar to that perceived for terrestrial diagenesis, namely, the excess free energy initially provided by thermodynamically-unstable minerals such as semi-amorphous clay minerals and high-temperature polymorphs¹; in the proto-planetary body, this may be provided by metastable phases^{23,24} formed after initial condensation (or re-condensation) in the solar nebula or in the interstellar medium. Eventually, water-ice liquefies (owing to heating and compaction) and lithification (owing to collapse of open-pore spaces) commences and, as the proto-planet evolves into a sub-planet, the loss of liquid water will be controlled by the sublimation rate of water-ice on the surface^{3,12}.

The present model is a credible scenario for low-temperature chemical reactions occurring in the intermediate stage of evolution between unaccumulated dust in the solar nebula and the formation of sub-planetary and planetary bodies. For example, hydrated silicates within interplanetary dust particles (IDPs)²⁵⁻²⁷ may have formed by the mechanism of interfacial water. IDPs are distinct extraterrestrial materials^{28,29} which may or may not be related to CI and CM carbonaceous chondrites²⁵, but their densities are much lower than those of the carbonaceous chondrites—1.5 to $<0.1 \text{ g cm}^{-3}$ (ref. 30) and 2.2–2.9 g cm^{-3} (ref. 31) respectively—and they are also more porous³². Nevertheless, both are considered relatively-primitive samples from early in the history of the Solar System^{31,33}. In a recent oxygen isotope study of the Murchison C2M meteorite, it was suggested that aqueous alteration in this meteorite have occurred at temperatures near the freezing point of water³⁴ and it seems possible that at least some low-temperature aqueous alteration in carbonaceous-chondrite parent bodies¹¹ have been initiated by the presence of interfacial water³⁵.

The early history of the Solar System is characterized by a period in which gravitational perturbations set newly formed bodies in collisional trajectories⁹ and it is conceivable that some proto-planetary bodies, or parts of them, may have been ejected into the outer Solar System where they evolved subsequently into secondary comets³⁶ and were stored at cryogenic temperatures. Solid material in the proto-planetary stage will be porous and of low density, whereas material from a sub-planetary body is likely to be less porous and of higher density. Thus I suggest that these arguments and the proposed model are consistent with the hypothesis that certain IDPs may be derived from proto-planetary material recovered from a cometary environment.

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Cathodoluminescence zoning and minor elements in forsterites from the Murchison (C2) and Allende (C3V) carbonaceous chondrites

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Although Mg-rich olivine occurs as isolated grains and in aggregates of carbonaceous meteorites¹, its chemical features have generally been neglected in favour of the more esoteric Ca/Al-rich inclusions with their challenging mineralogy and isotope systematics. Because cathodoluminescence has proved useful in revealing textures and compositional varieties for some minerals in meteorites, we have now applied the technique to look for textural features of olivine in carbonaceous meteorites relevant to the unresolved dispute over its origin, whether from a vapour or a liquid²⁻⁹. Our cathodoluminescence photographs of forsterite grains in Murchison (C2) and Allende (C3) meteorites reveal a blue core (inclusion-free) with planar boundaries to a red or dark rim (inclusions of glass, metal and silicate). We have also performed high-precision electron microprobe analyses revealing in these forsterites unusually large amounts of the 'minor' elements Al, Ti and Ca in the blue cores (and sharp chemical changes at the blue/red boundary), suggesting formation by crystallization at high temperatures from a source rich in these metals. Following conclusions drawn from previous analyses of olivine in meteorites (achondrites¹⁰, Niger (I) C2⁷, Belgica 7904 (C2)¹¹), we believe the minor element signature should be able to characterize olivines in micrometeorites and in deep-sea particles (see accompanying paper¹²).

The dispute about the origin of olivine in Murchison is based mainly on textural evidence²⁻⁹. Olivines occur with up to 80% Fe_2SiO_4 (fayalite)², and we consider here only the forsterites, most of which occur as isolated grains within the matrix. There are many textural types of olivine in the Allende meteorite, which has complex petrographic features like other members of the Vigarano type¹³. The present study considers only the isolated Mg-rich olivines, and does not consider the range of olivines in the amoeboid aggregates¹⁴ and exotic components¹⁵.

All Mg-rich forsterites in both meteorites exhibit cathodoluminescence and contain substantial amounts of several trace elements (Mn, Ti, Cr), which may be activators. The best examples of cathodoluminescence zoning occur in the Allende olivines (Fig. 1). The important textural features are: (1) blue-luminescent olivine occurs as cores of isolated subhedral-to-anhedral single crystals (Fig. 1a); (2) each rim is continuous and usually shows a weak red cathodoluminescence which vanishes at the boundary with the matrix; (3) in some crystals, the red-luminescent olivine abuts the matrix without an intervening dark outer rim; (4) dumbbells and clusters of grains are common, with each blue core surrounded by its own red rim; (5) most of the boundary of each blue core is composed of straight contacts with the red zone, although a completely euhedral boundary was not found (Fig. 1a, b); (6) an irregular network of red or dark olivine bands, some of which are curved (Fig. 1b) and some straight, traverses some of the blue cores; (7) inclusions of metal or glass are rare in blue cores, but common in dark regions. A particularly striking variety (Fig. 1c) is an almost round blue olivine enclosed by another blue grain interpenetrated by bright-blue fine-grained material (possibly devitrified glass).

In the Murchison meteorite, most of each olivine grain exhibits blue cathodoluminescence whereas the red dark rim is usually incomplete. Each grain is sharp and angular, and there is no evidence from the cathodoluminescence for reaction between the olivine and the matrix. In reflected light, the surface

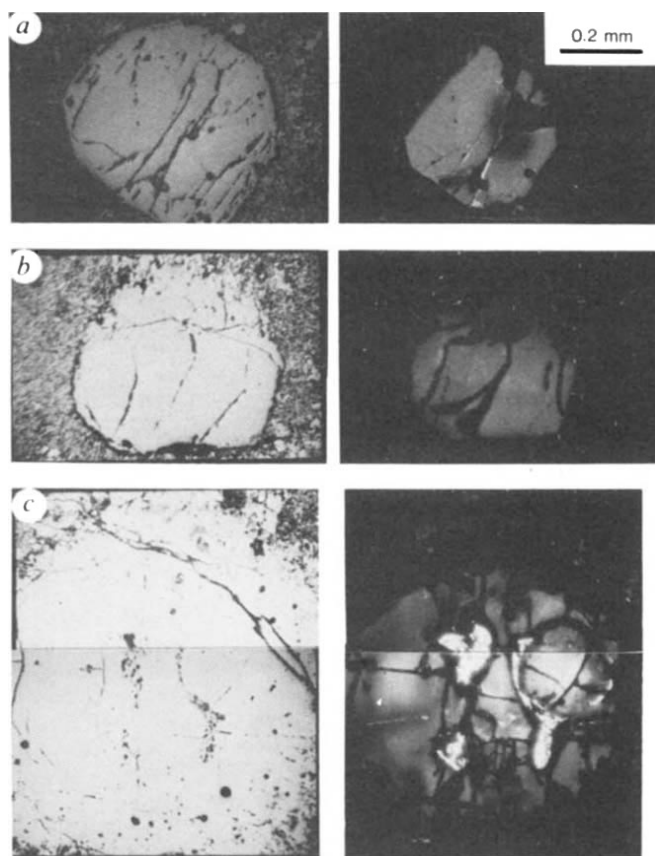


Fig. 1 Pairs of photographs in reflected light (left) and cathodoluminescence (right) of three forsterite crystals from the Allende meteorite. The black and white rendition of the cathodoluminescence allows distinction only between blue cathodoluminescence (bright) and dull red or no cathodoluminescence (dark). All photographs have the same scale. *a*, Subhedral luminescing core showing embayments (upper right) filled with non-luminescing olivine: euhedral boundaries of the core (right) correspond with the external boundaries of the non-luminescing rim (left). *b*, Subhedral luminescing core with apparent fractures filled with non-luminescing olivine: the upper portion of the core shows brighter cathodoluminescence than the lower portion, with a sharp boundary. *c*, Luminescing core with erratic variation of cathodoluminescence: sub-round included luminescing grain; network of fractures filled with non-luminescing olivine, and areas filled with fine-grained brightly luminescent material, possibly glass.

of the Murchison olivines displays a fretted pattern even though the blue cathodoluminescence is quite uniform. Only one distinct mineral inclusion, a pure MgAl_2O_4 spinel, was found in the blue olivine.

Olivine analyses were performed in conditions given in Table 1 of the accompanying paper¹². Figure 2 shows characteristic profiles of the composition of olivine grains from Allende and Murchison. The step scan from a blue core of the Allende olivine to its red inner rim shows a sharp change of Al content near the cathodoluminescence boundary, whereas Ca and Ti begin to decrease well within the blue core; Fe increases mainly in the outer rim, and Mn changes somewhat erratically. The Murchison olivine grain is unusual because it is rounded rather than angular, and shows concentric zoning; it was selected because the angular grains showed truncation of the zoning. The core has greater amounts of Ca, Ti and Al than the rim, and less Cr, Fe and Mn.

A clear distinction between the Murchison and the Allende olivine grains having blue luminescent cores is the smaller range of FeO (0.3–1.4 wt%) in the former (Fig. 3). Although most of the Allende olivines with a blue core have ~0.5 wt% FeO, there

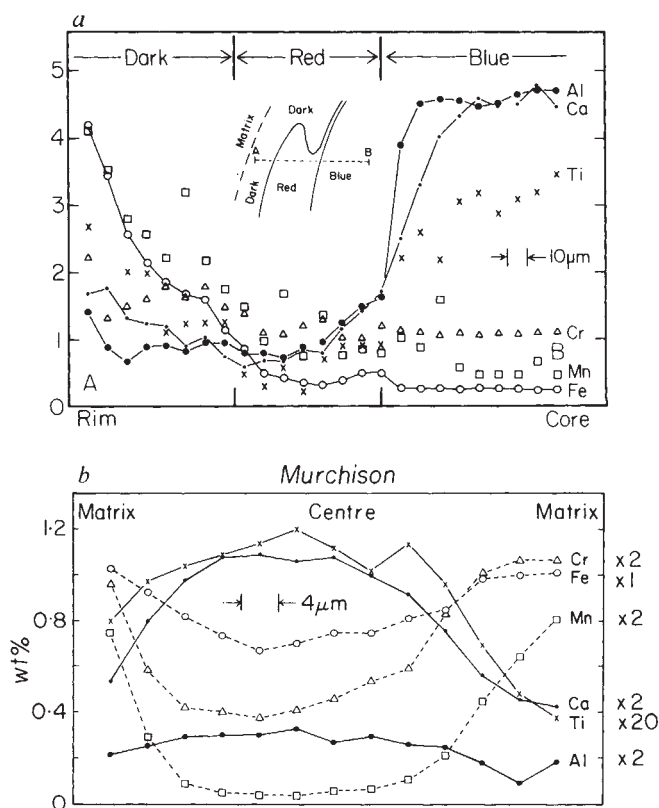


Fig. 2 Profiles for Al_2O_3 , CaO , TiO_2 , Cr_2O_3 , MnO and FeO across cathodoluminescence zones (inset): *a*, forsterite crystal from the Allende meteorite; *b*, forsterite crystal from the Murchison meteorite.

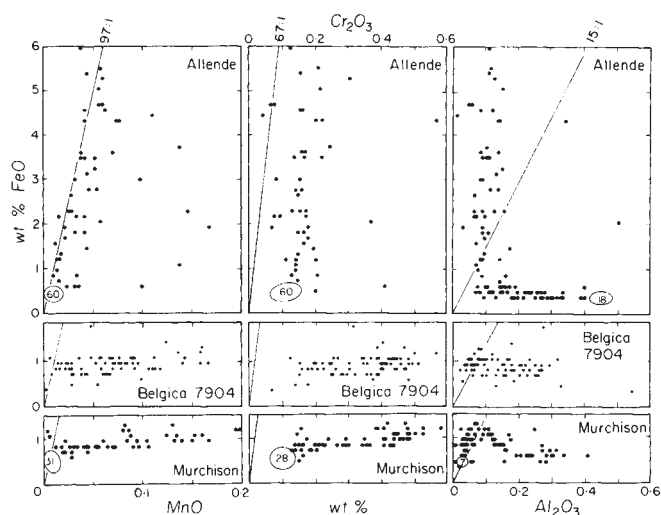


Fig. 3 Relation between FeO and MnO , Cr_2O_3 and Al_2O_3 in isolated forsterite crystals from the Allende, Belgica 7904 and Murchison meteorites. Each point represents one electron microprobe analysis; clusters of adjacent points are represented by an enclosed number.

is a continuous band reaching 6 wt%. Furthermore, the Murchison olivines display a larger range of Cr_2O_3 (0.1–0.6 wt%) and MnO (0–0.2 wt%) than the main band of Allende olivines. (We cannot explain the few scattered analyses for Allende olivine.) Alumina ranges up to 0.4 wt% in olivines from both meteorites, but the FeO – Al_2O_3 patterns are quite distinctive. The Belgica 7904 meteorite¹¹ shows similar ranges in composition to Murchison, but lacks the cluster of analyses near FeO

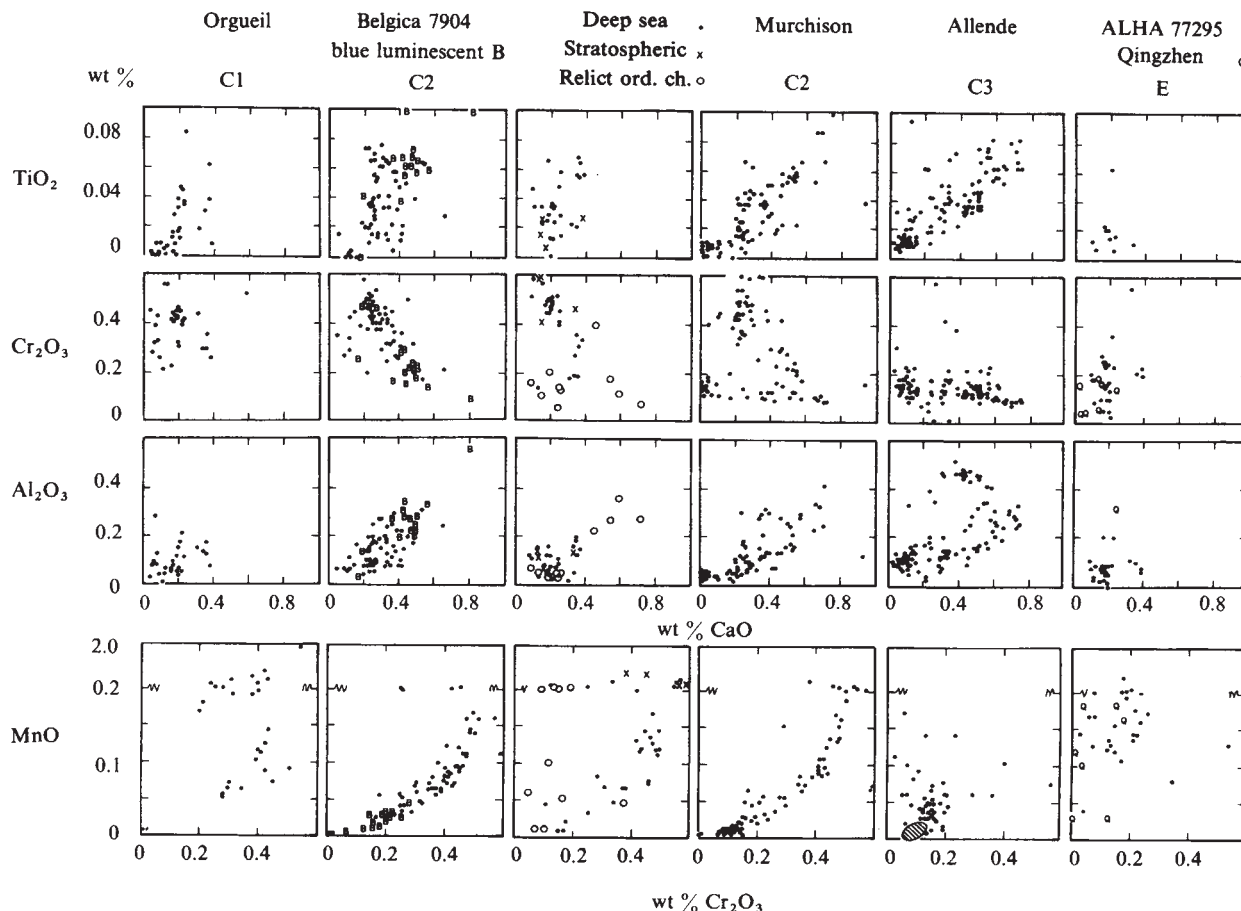


Fig. 4 Compositional relations in forsterites. Each point represents one analysis except in crowded regions (the shaded area contains about half the MnO versus Cr_2O_3 analyses in Allende). Sources: these are new analyses except those for relic olivines in ordinary chondrites¹⁷ and olivines in Qingzhen³¹. The analyses for olivines in deep-sea particles are given in ref. 12. The four analyses for two stratospheric particles will be published when further data have been collected.

0.5, MnO 0.01, Cr_2O_3 0.1 and Al_2O_3 0.03 wt%. Detailed study of the C2 meteorites is in progress to establish the nature of the subpopulations of forsterites. In the Allende olivines, the L-shaped distribution on the FeO - Al_2O_3 plot is split into a blue population (low Fe, low-to-high Al) and a red population (low Al, low-to-high Fe). Correspondingly, in the C2 meteorites the blue olivines also have a higher Al_2O_3 content than the red ones. Alumina is correlated with TiO_2 and CaO, and Ti may be a major control on the cathodoluminescence colour.

Figure 4 compares the compositional properties of forsterites from Orgueil (C1), Belgica 7904 and Murchison (C2), Allende (C3), Qingzhen¹⁶ and ALHA 77295 (E), and deep-sea particles, stratospheric particles and relic cores of Fe-rich olivines in unequilibrated ordinary chondrites¹⁷. The data for all olivines except Qingzhen and the ordinary chondrites are of high precision and were measured by the same technique. Those for Qingzhen and the ordinary chondrites (Krymka, Chainpur and Bishunpur) are of unspecified precision, and are probably of normal accuracy.

The olivines from the different meteorite families can be differentiated on selected two-dimensional plots (for example, MnO versus Cr_2O_3 and CaO versus TiO_2 , Cr_2O_3 or Al_2O_3), and their resolution is further improved in multidimensional space. The MnO- Cr_2O_3 plot separates the E meteorites from the C meteorites; the positive correlation for the two C2 meteorites (Murchison, Belgica 7904) is particularly good and similar to that for the deep-sea particles. Further conclusions should follow as the database increases.

High levels of Al and Ti, a positive correlation with high levels of Ca, and significantly high levels of Cr⁷, are unique

features of those luminescent olivines found in the Allende and Murchison meteorites. All terrestrial forsterites with high Ca come from high-temperature rocks¹⁸; the highest Al_2O_3 is only 0.10 wt%, in forsterites from high-temperature peridotite xenoliths from kimberlites¹⁹. A maximum of 0.5 wt% TiO_2 occurs in forsterites from dunite xenoliths in kimberlites²⁰. All high-precision analyses of TiO_2 in achondritic meteorite olivines are lower in TiO_2 than the upper limits for TiO_2 in the forsterites in the Murchison and Allende meteorites¹⁰. In the range of lunar rock olivines²¹ with 91–45% forsterite component, Al_2O_3 reaches 0.15 wt% in a granulitic impactite, but is mostly below 0.04 wt% in other rocks; TiO_2 , however, rises to 0.22 wt% in another granulitic impactite and the median value of 0.04 wt% is in the middle of the ranges for the Murchison and Allende meteorites. In terrestrial forsterites, the highest value of 0.4 wt% Cr_2O_3 for an olivine in dunite²² is less than the maximum of 0.6 wt% for Murchison olivines and 0.8 wt% for Niger (I) olivines⁷. We conclude that the Allende and Murchison forsterites crystallized at high temperature from sources rich in refractory elements—indeed, V is also detected at the 100 p.p.m. weight level in blue Allende olivine.

Whereas most of the Allende forsterites lie fairly close to the cosmic ratio of 97:1 for FeO/MnO ²³, the Murchison forsterites lie in a band in which the ratio changes from close to the cosmic value for the blue cores to only six for the extreme values in a dark rim (Fig. 3). Although crystal-liquid and crystal-crystal partition coefficients for Fe^{2+}/Mn differ from unity^{21,24–26} for basaltic-peridotitic compositions, and although there is some variation of FeO/MnO among the olivines of achondritic meteorites¹⁰, the change of Fe/Mn in the Murchison forsterites

is too large to be explained by a simple crystal-liquid fractionation for an oxidized chondritic bulk composition. However, the very low Ni content in the Allende and Murchison forsterites implies that the Fe was predominantly in a reduced state during crystallization of the forsterites; further experimental work is needed on Fe/Mn partitioning in such conditions.

The sharp compositional gradients, especially for the refractory elements Al, Ca and Ti, suggest that chemical properties of the forsterites are essentially the same now as at their inception. We tentatively suggest an investigation of (1) the growth of each blue core from a vapour rich in refractory elements (hence the paucity of inclusions, and the minor-element chemistry), and (2) the growth of the red/dark rim from a liquid that trapped the blue core. Mechanical transfer may be involved within an inhomogeneous medium. Crystallization of Ca, Al, Ti-rich phases may have changed the composition of a reservoir between the growth of a blue core and that of a red/dark rim. Survival of olivine grains during formation of chondrules is now well documented^{17,27-31}, and forsterite grains survive the melting of micrometeorites to yield deep-sea particles³². There is no direct relationship between the textures and minor-element chemistry (Fig. 4) of the Murchison and Allende zoned olivines with those of relic olivines in the ordinary chondrites^{17,27-30} and the ALHA 77295 and Qingzhen E3 chondrites³¹; nor is there a complete chemical match with the blue and orange luminescent forsterites in the Indarch and Kota-Kota enstatite chondrites³³.

These differences preclude a common origin for the forsterites in the various types of chondrites studied so far, and demonstrate the value of the chemical signature as a genetic guide, for example, for characterization of deep-sea particles¹². Second, the complex textural and chemical features, including embayments and networks of dark olivine within blue cores, point to multi-stage events, and place constraints on the environment during growth, melting and further crystallization. Further studies involving isotopic analyses as well as textural and chemical measurements will test whether these complex processes occur only in the solar nebula, or whether they relate in part to interstellar dust^{34,35}.

We thank NASA for grant NAG 9-47; E. Olsen (Field Museum) for supply of the meteorites; N. Weber and O. Draughn for technical assistance, and J. F. Kerridge for helpful criticisms.

Minor-element signature of relic olivine grains in deep-sea particles—a match with forsterites from C2 meteorites

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Deep-sea particles (DSP) extracted magnetically from oceanic sediments are the products of aerodynamic heating and cooling of extraterrestrial materials¹. Most DSP have bulk compositions and elemental ratios similar to CI and CM(C2) carbonaceous meteorites² and some contain relic grains³ of Mg-rich olivine (forsterite). Here we describe the results of high-precision electron microprobe analyses on relic forsterites (26 spots) in 13 different DSP, comparing their 'minor'-element (Mn, Cr, Ca, Ti, Al) signatures with those of olivines found in several types of meteorites. Our studies show that the DSP olivines do not match those from terrestrial and lunar rocks and achondritic meteorites⁴; match poorly those of the carbonaceous Allende (CV3) and Orgueil (CI) meteorites⁵; but are well matched with olivines from C2 meteorites^{6,7}, including the unusual Belgica 7904 meteorite⁷. We believe this demonstrates the power of the minor-element signature to fingerprint olivines—an idea we have introduced in the accompanying paper⁸.

The deep-sea particles contain much olivine, but most olivine is Fe-rich and has a fine texture attributed to rapid crystallization from a melt created during entry into the atmosphere⁸. Each particle selected contains at least one relatively large olivine grain of almost pure forsterite (Mg-rich olivine) which may be overgrown by relatively Fe-rich olivine—the boundary between is abrupt. The bright blue cathodoluminescence of the forsterite is distinctive by the absence of luminescence in the olivine with more than 2% Fe₂SiO₄. The results of the electron microprobe analyses are given in Table 1.

There was no match between the minor-element signature of the relic olivines in DSP and olivines from terrestrial rocks. First, almost pure forsterite is very rare in terrestrial rocks, only occurring in some metamorphosed impure limestones⁹. Second, terrestrial forsterites¹⁰, from igneous rocks, have typically 0.4 wt% NiO, in contrast to the low values (near the detection limit) of NiO in DSP relic olivines (Table 1). Third, the levels of Cr₂O₃ (0.12–0.48, mean 0.39 wt%), TiO₂ (0–0.07, mean 0.03 wt%) and Al₂O₃ (0.01–0.19, mean 0.09 wt%) in the DSP relic olivines are not matched simultaneously by terrestrial olivines. This direct mineralogical evidence confirms the earlier conclusion^{1,2} from bulk chemical and isotopic analyses that DSP originate from an extraterrestrial source.

Lunar olivines¹¹ do not provide a match, particularly for Fe and Mn. Olivines in pallasites, ureilites, nakhlites, shergottites, Brachina and Chassigny contain too much Fe⁴. The obvious possible match is with olivines from carbonaceous chondrites: many studies¹² have shown that forsterite (0.5–5.0 Fe₂SiO₄) is abundant in all three principal groups. Because the data for minor elements in these forsterites are not comprehensive, we supplemented the existing data (for example, Niger I (C2)⁶, Belgica 7904 (C2)⁷) with new measurements (Murchison (C2)⁵; Allende (CV3)⁵; Orgueil (CI), Mighei (C2), in preparation). We are not concerned here with the controversial origin of the forsterites⁵ but only with their minor-element signatures.

Figure 1 shows the correlation between FeO and MnO, which is useful for sorting achondritic olivines. The positive trend for DSP forsterites and those for the three C2 meteorites match reasonably well, but new data of higher precision are needed for Niger (I)⁶. Data for the forsterites from the Allende CV3 meteorite do not match the trend; the relatively high Ni in the

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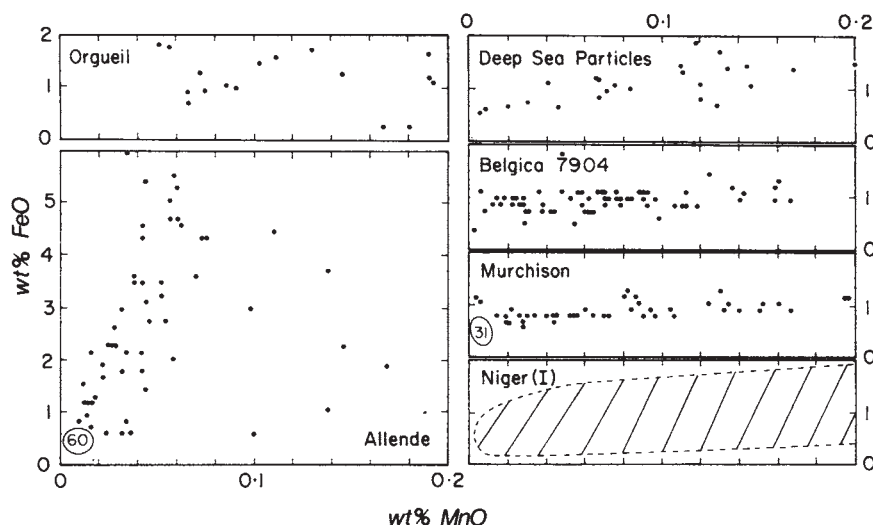


Fig. 1 MnO versus FeO for relic olivines in deep-sea particles (Table 1) and isolated Mg-rich olivines in the Orgueil (C1), Allende (CV3)⁵, Murchison (C2)⁵, Belgica 7904 (C2)⁷ and Niger I (C2)⁶ meteorites. Each point represents one analysis. Clusters for Allende and Murchison are shown by ringed numbers. Analyses for Niger I were taken from Fig. 7 of ref. 6: most analyses fall within the hatched region; the standards and precision were not given. Many Orgueil olivines have more than 2 wt% FeO, and all have a higher Ni content than the olivines in C2 and C3 meteorites.

Orgueil olivines precludes a match with these data, no matter how well the Mn and Fe compare. Figure 2 compares the ranges of olivine compositions in two of the C2 meteorites, and suggests that the DSP relic olivines match more closely the olivines from Belgica 7904 than those from Murchison. Most of the olivines lie in a band from 0.5 wt% Cr₂O₃ and 0.2 wt% CaO to 0.2 wt% Cr₂O₃ and 0.5 wt% CaO. Twenty-two analyses for DSP relic olivines lie in the band and three lie on the edge of the band. Some olivines from Murchison appear to fall into a second band with 0.15 wt% Cr₂O₃ and variable CaO. Only one of the analyses of the DSP relic olivines lies in this second band, but it must be emphasized that there are only 13 relic olivines and hence statistical control is insufficient.

Although the present sample of minor-element signatures is small for both the DSP relic olivines and the forsterites from carbonaceous meteorites, the general match with the C2 olivines is striking. This reinforces the similarity already found³ between the major-element ratios of bulk DSP and C2 meteorites. The presence of diopside-rimmed spinel-perovskite aggregates in some DSP¹³ also suggests a match with C2 meteorites¹⁴. Incidentally, any Fe-rich olivines from a C2 source should melt more easily than the forsterites because of the general decrease in melting temperature that occurs with increasing Fe/Mg ratio.

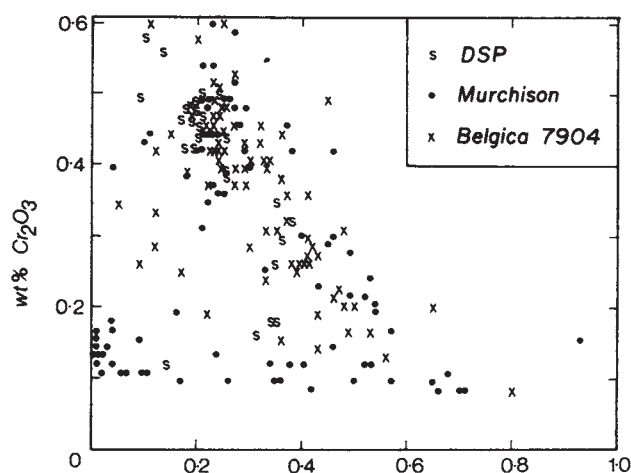


Fig. 2 CaO versus Cr₂O₃ for relic olivines in deep-sea particles (DSP) (Table 1) and isolated Mg-rich olivines in the Murchison and Belgica 7904 (C2) meteorites^{5,7}.

Table 1 Electron microprobe analyses of relic olivines in deep-sea particles

	KK1Q-8			KK1Q-185			KK1Q-100		KK1T-7		KK1T-10		KK1T-12
	1	2	3	1	2	3	1	2	1	2	1	2	1
Al ₂ O ₃	0.069	0.078	0.060	0.172	0.154	0.190	0.142	0.120	0.010	0.039	0.115	0.062	0.155
CaO	0.210	0.185	0.202	0.350	0.348	0.350	0.381	0.362	0.314	0.343	0.212	0.178	0.201
TiO ₂	0.032	0.027	0.033	0.067	0.054	0.053	0.056	0.063	0.022	0.019	0.024	0.024	0.012
Cr ₂ O ₃	0.502	0.478	0.438	0.347	0.259	0.180	0.317	0.292	0.158	0.178	0.462	0.464	0.476
MnO	0.144	0.128	0.119	0.046	0.030	0.020	0.067	0.083	0.008	0.005	0.075	0.071	0.168
FeO	1.09	0.718	0.810	0.670	0.747	0.690	0.857	1.01	0.625	0.564	1.08	0.960	1.38
NiO	0.018	0	0	0.005	0.009	0.031	0.016	0.013	0	0	0.006	0	0.042
	KK1T-12	KK1T-13	KK1T-10	KK1K-15	2	KK1K-9	KK1K-10	2	3	KK1J-9	2	KK1J-5	2
	2	1	1	1		1	1			1		1	
Al ₂ O ₃	0.109	0.145	0.041	0.013	0.029	0.031	0.033	0.083	0.064	0.113	0.102	0.101	0.079
CaO	0.199	0.141	0.197	0.214	0.209	0.147	0.255	0.257	0.246	0.097	0.106	0.257	0.189
TiO ₂	0	0.021	0.011	0.033	0.035	0.034	0.014	0.028	0.047	0.045	0.034	0.057	0.065
Cr ₂ O ₃	0.422	0.561	0.489	0.472	0.453	0.118	0.381	0.386	0.500	0.494	0.576	0.435	0.422
MnO	0.201	0.364	0.118	0.133	0.142	0.041	0.067	0.666	0.118	0.110	0.210	0.116	0.129
FeO	1.51	0.899	1.10	1.41	1.44	1.11	1.21	1.22	1.38	1.33	1.49	1.88	1.72
NiO	0.007	0.013	0	0.013	0.013	0.007	0.008	0	0.013	0	0.019	0.024	0.048

Analytical conditions: standards—P140 olivine (Mg, Si, Fe), enstatite glass with 5% Al₂O₃ (Al), diopside glass (Ca), Corning V-glass (Ti, Cr), Corning W-glass (Mn), Corning X-glass (Ni); 25 kV; 100-nA sample current on forsterite; 10-μm beam diameter; backgrounds measured for each spot; detection limits (2 s.d.)—Al₂O₃ (0.022 wt%), CaO (0.008), TiO₂, Cr₂O₃ and MnO (0.007), FeO and NiO (0.014); ZAF (atomic number, absorption, fluorescence) correction; count rates for Mg and Si (30,000 counts s⁻¹) too high for accurate deadtime correction, but suitable for identification and ZAF correction.

Detailed analyses of forsteritic olivines in additional DSP and carbonaceous meteorites are in progress to see whether a minority of the DSP olivines can be matched reliably with olivines from C1 and C3 meteorites—also whether the majority of DSP olivines can be further matched with the subgroup of the C2 meteorites. Detailed study of the trace elements and textural features of the olivines in DSP and other extraterrestrial materials should impose additional constraints on their origin. Presently, it is important to recognize that precursors of DSP are dominated by carbonaceous chondritic material whereas macrometeorites mostly consist of ordinary chondrites.

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A charge-decoration technique for studying the heterogeneity of coexistent monolayer phases by electron microscopy

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Phospholipid or fatty acid monolayers at the air-water interface exhibit a series of pressure and temperature-dependent two-dimensional conformational changes¹⁻³. New techniques¹⁻³ of studying the microscopic heterogeneity of lipid monolayers have shown that, with increasing pressure, they undergo first a gas-fluid phase transition via a two-dimensional foam², and subsequently a transition, extending over a finite pressure region of several mN m⁻¹, from the fluid to a crystalline state. This could be attributed to the coexistence of these states¹ and has been explained in terms of their different electric polarizations—a new picture of the fluid-solid phase transition. We have demonstrated⁴ that monolayers may be transferred from the air-water interface to solid substrates, not only from completely condensed, but also from more expanded, states such as these fluid-solid coexistence regions of pure and mixed monolayers. This suggests new possibilities of preparing Langmuir-Blodgett films with mosaic-like arrangements of crystalline and amorphous patches several micrometres in diameter⁵. We report here a new electron microscopy technique which allows us to study the microstructure of such films, without any staining, up to a resolution of 1,000 Å. Different phases may be distinguished by phase contrast, owing to differences in electric charging caused by a corresponding difference in the electric conductivities, and electron diffraction patterns taken from selected areas can give information on the molecular packing of the phases.

Our ability to transfer monolayers from expanded states, without severe alteration to the structure, suggests new applications in molecular electronics⁶, integrated optics⁷ and as models

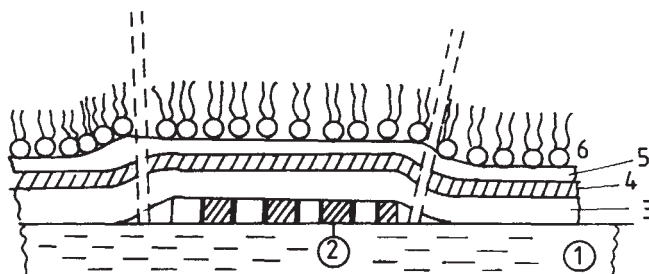


Fig. 1 Sandwich-type substrate for transfer of the monolayers from the air-water interface, comprising an electron microscopy grid (2), covered with a Formvar film 200 Å thick (3), a carbon film 50 Å thick (4), and a silicon monoxide layer 20 Å thick (5). The carbon film provides a conducting layer and the silicon monoxide, a well-defined hydrophilic surface. The grids are deposited on a cover slip (1) to reduce edge effects which destabilize the monolayers (6) if they are directly transferred on substrates as small as an electron microscope grid. The transfer is achieved by the Langmuir-Blodgett method by pulling the whole substrate out of the water, at constant pressure and at a speed of 0.5 cm min⁻¹. For observation, the grid is detached from the coverslip as indicated by the dashed lines.

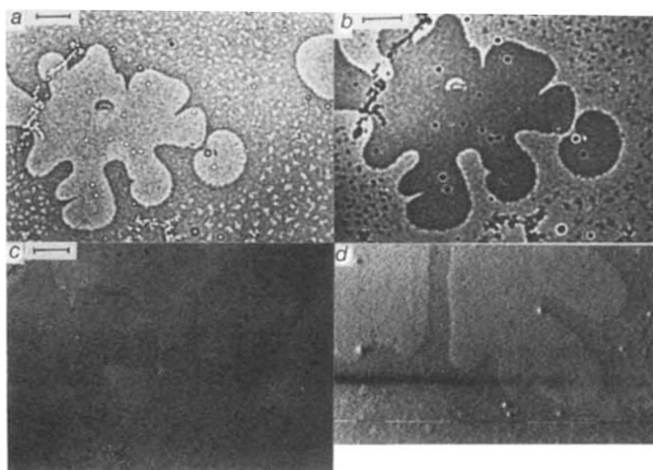


Fig. 2 Electron micrographs of a pure dimyristoylphosphatidic acid (DMPA) monolayer transferred from the fluid-solid coexistence region. (Scale bars 5 µm.) a-c, Low-magnification phase-contrast micrographs taken at different degrees of defocusing: a, overfocus of $f = +10$ mm; b, underfocus of $f = -10$ mm; c, underfocus of $f = -1$ mm. In a parallel electron diffraction experiment, it has been shown that the quasi-dendrite platelets exhibit hexagonal diffraction patterns and thus form two-dimensional crystallites whereas the residual area is in an amorphous state. The small dark or bright spots represent holes in the Formvar foil; the actual size of these holes is much smaller (0.1-0.5 µm). d, Scanning transmission micrograph, in the dark-field mode, of a DMPA monolayer in the same state as in a-c. By measuring the intensity of the elastically scattered electrons it has been shown that the mass density of the crystalline monolayer dendrites is greater by ~25% than that of the amorphous patches. Actually, the relative mass difference is 1.4% because of the contribution of the substrate which was estimated by calibration with carbon layer exhibiting a step of well defined height. The rather high contrast of the micrograph occurs because part of this background intensity has been subtracted electronically. The small spots are again caused by holes in the Formvar film and they exhibit shadows because of the nonlinear response of the amplifier.

of biological membranes⁸. For example, amphipathic membrane proteins with polar head groups and a hydrophobic core, such as the insulin receptor, may be incorporated into a fluid monolayer but are expelled from condensed crystalline states (W. Pfeiffer and E. Sackmann, unpublished work). By transferring from a fluid-solid coexistence region, the proteins may remain in the fluid patches while the protein-free crystalline part provides for a high mechanical stability—an important step

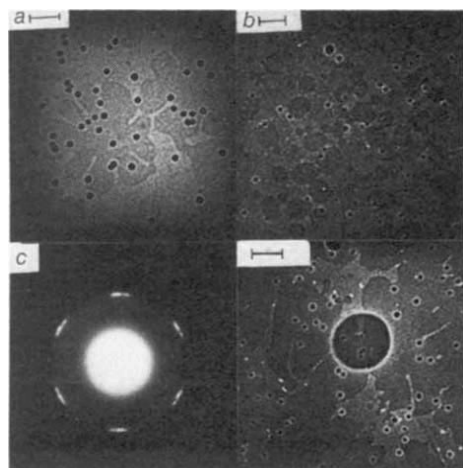


Fig. 3 Phase-contrast electron micrograph of a mixed monolayer of DMPA containing 10 mol % (a) and 20 mol % (b) of cholesterol (scale bar 5 μm). The film was deposited at a constant pressure of 30 mN m^{-1} at 20 $^{\circ}\text{C}$. According to film-balance experiments, the monolayer is completely condensed at this pressure. c (Left), An electron diffraction pattern taken from the area indicated by the dark disk on the micrograph on the right, which defines the area probed by the electron beam. A superposition of two mutually rotated hexagonal diffraction patterns is observed, showing that the dark domains correspond to a crystalline monolayer phase of almost pure DMPA¹¹. Two diffraction patterns are observed because the electron beam passes an area where two platelets, of slightly different orientation of the crystal planes, coalesce. No sharp reflections are observed if the electron beam traverses only the bright regions. From platinum-shadowing experiments it follows again that they are covered by a monolayer and thus correspond to an amorphous phase, rich in cholesterol.

towards the preparation of biosensors by combining Langmuir-Blodgett films with field-effect transistors. Transfer of expanded lipid monolayers to small substrates such as electron microscope grids covered with carbon films is not possible because edge effects cause rupture of the monolayer during the transfer process and so we have used the rather complex substrate, shown in Fig. 1, which provides a large area to minimize edge effects and which allows only one side of the substrate to be covered. For electron microscopy the electron microscope grid can be detached from the glass plate. The microstructure of the monolayer on the air-water interface can be observed, to a resolution of several micrometres, by a recently developed microfluorescence technique² and it is therefore possible to test whether the transfer has been accomplished without severe structural changes.

The electron micrographs are taken with a Philips EM 400T microscope, in the low magnification mode, at a very small angle of illumination. A magnetic shutter above the specimen allows one to work with a minimum dose. High beam coherence, necessary for image formation, is achieved by using a highly energized first condenser lens and a large focal length of the objective lens. The electron optical configuration is similar to that for the Lorentz microscopy of ferromagnetic domain walls⁹, allowing, in particular, large values of defocusing (up to several millimetres) of the objective lens to be achieved.

The contrast formation is demonstrated in Fig. 2 for a dimyristoylphosphatidic acid monolayer (DMPA) transferred at a pressure slightly above the fluid-solid transition: the quasi-dendrite platelets are two-dimensional single crystals distinguished by their sharp diffraction spots (compare with Fig. 4b). Although the residual areas do not exhibit an observable diffraction, they are definitely covered by a monolayer, as revealed by platinum shadowing experiments showing the difference in height between the two phases to be certainly $<10 \text{ \AA}$ and also by darkfield-scanning transmission electron microscopy experiments¹⁰ (compare with Fig. 2d) which measure the relative cross-section of elastically scattered electrons, showing the

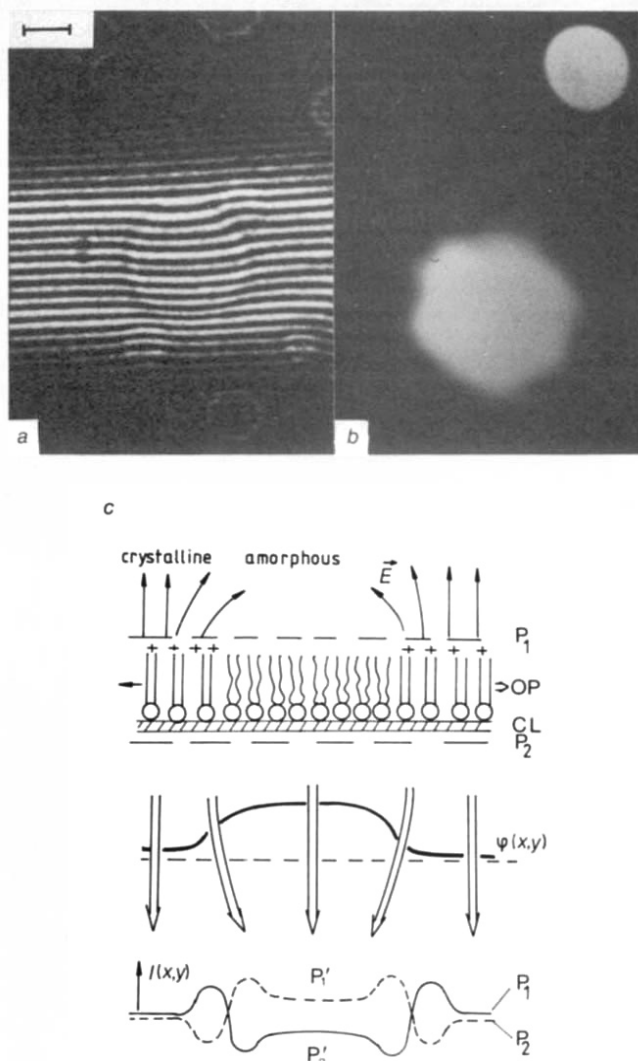


Fig. 4 a, Micrograph of the monolayer of a mixture of arachidic and perfluorodecanoic acids taken with a microscope equipped with an interferometer. The film was transferred from the fluid-solid coexistence region. A diffraction experiment shows that the almost circular patches correspond to the fluid phase and the residual area is covered by a crystalline monolayer. The interference fringes of the two phases are mutually shifted by about one fringe distance, corresponding to a phase shift of $\sim 2\pi$ (Scale bar 1 μm). Note that the shifts associated with the larger and the smaller circular domains are in opposite directions because the interferometer used is a Fresnel-type biprism allowing only relative phase shifts between the two half waves to be measured. Thus, if two platelets are situated at opposite sides with respect to the symmetry axis, they produce opposite phase shifts. b, Demonstration of small-angle deflection of the electron beam by the heterogeneous monolayer of a transferred to pure carbon substrate. The aperture of the undisturbed beam is $0.8 \times 10^{-5} \text{ rad}$ (disk in upper right-hand corner). The broadening caused by the monolayer (lower bright spot) corresponds to an angle of deflection of $2 \times 10^{-5} \text{ rad}$. c, A schematic representation of contrast formation by charge decoration. Top trace, monolayer with excess charges producing an excess electric field, E . Also shown are the object plane (OP) and two situations of defocusing, where P_1 and P_2 denote the image planes. Note that the electric field at the bottom of the specimen is compensated by the conducting carbon layer (CL) of the substrate. Middle trace, wavefront of the electron beam $\phi(x, y)$ distorted by the excess electric field, E . The vertical component of E leads to a phase shift and the horizontal component to a simultaneous deflection of the electrons, which is largest at the boundaries between the phases. Lower trace, schematic representation of the electron distribution at planes P_2 and P_1 : note that P_2 indicates the real electron distribution at the plane P_2 below the specimen, and P'_1 the virtual distribution at the plane P_1 , above the specimen.

relative difference in mass density of the two types of textures to be 25% of a monolayer. This corresponds well with the change in packing density of $\sim 30\%$ observed at the fluid-solid transition. Thus, the heterogeneous monolayer can indeed be transferred from the fluid phase to solid substrates without substantial structural change.

An example of a heterogeneous monolayer for a mixture where both phases are in completely condensed states is shown in Fig. 3 with the relative area covered by the dark domains decreasing from 70% in *a* to 30% in *b*. The domains are thus formed by a crystalline solution of cholesterol in DMPA, creating two-dimensional single crystals (Fig. 3*c*). The bright areas do not exhibit sharp reflections and thus correspond to an amorphous cholesterol-rich phase, in complete agreement with a phase diagram for this mixture (established previously by film-balance techniques¹¹) which exhibits a miscibility gap in the solid state of the phosphatidic acid.

The following observations provide evidence that the contrast between the crystalline and the amorphous monolayer is caused by a different electric charging of these two phases. (1) The contrast appears gradually after irradiating the sample with a dose of 10^{-3} – 10^{-4} electrons $\text{\AA}^{-2}$ and is accompanied by secondary electron emission. The charging of the monolayer is retained, and thus the contrast maintained, for some minutes after switching off the electron beam. (2) All micrographs shown were taken before any beam damage was observable. The contrast decreases after prolonged exposure, accompanied by a simultaneous decrease in the diffraction intensity. Such radiation damage becomes noticeable at doses > 1 electron $\text{\AA}^{-1}$. (3) The contrast disappears if the lipid monolayer is covered by a conducting platinum layer; it is attenuated gradually if carbon layers of increasing thickness are deposited. (4) The electron waves are both shifted in phase and deflected on transversing the heterogeneous monolayers, as is demonstrated in Fig. 4*a* and *b*, respectively.

The contrast formation may be explained in terms of the charging of the monolayers as described in Fig. 4*c* for the case of crystalline domains carrying an excess positive charge whose associated electric field is not compensated above the specimen and exhibits both a vertical component and a horizontal component which is strongest at the boundaries between the phases. One therefore expects a phase-shift difference between the waves traversing the two phases as well as a deflection of the electrons, with both effects producing a contrast in the case of defocusing¹². In the case of focused images a contrast can also be produced if part of the low-angle deflection is suppressed by insertion of an aperture (A. Fischer, in preparation). However, we cannot presently account quantitatively for the large phase shift of $\sim 2\pi$: it cannot stem from the mass difference of the monolayer phases, as even a 200- $\text{\AA}$ -thick carbon layer would give a shift of only $\pi/2$, but must arise from an electric field extending far into the space above the specimen.

We have demonstrated that the charging of isolated lipid monolayers on conducting substrates provides a simple method for distinguishing different phases of transferred lipid monolayers by transmission electron microscopy, with simultaneous electron diffraction providing information on the local molecular structure. On the other hand, monolayers can be easily transferred from the air-water interface to the substrate without structural changes at least at pressures above the liquid-solid transition of pure, or above the liquidus line of mixed, monolayers. Therefore, the present technique can provide a powerful method for studying the microscopic structure of heterogeneous lipid monolayers. We will show elsewhere that it may also be exploited to establish phase diagrams for mixed monolayers.

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Enrichment of ^{235}U and the concentration of ^{239}Pu in volcanic samples

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There have been at least two reports^{1,2} of volcanic materials containing enhanced levels of plutonium. In both cases, the Pu/U ratio ranged from 10^{-10} to 10^{-8} , compared with a ratio of 10^{-12} in uranium ores of non-volcanic origin³. The natural occurrence of significant concentrations of plutonium in the Earth's crust or mantle would have profound implications for many geophysical processes, but all the samples analysed so far were collected after the first nuclear explosions, and thus may have been contaminated by atmospheric fallout. We have therefore now measured the plutonium content and uranium isotope ratio of a number of samples collected before the first nuclear explosions. The results conform with those reported for non-volcanic uranium ores.

The potential causes for changes in the Pu/U ratio include the possibility of selective migration of the small amounts of Pu normally found in uranium minerals to areas of low uranium abundance. Should the volcanic eruption cause the Pu-rich fraction to be selectively sampled, the lava would appear to have a high Pu/U ratio. A similar effect would be found if the uranium is removed selectively either before or during the eruption.

If the Pu-rich strata had been undisturbed for a period of time that was long compared with the half life of ^{239}Pu (24,400 yr), the ^{235}U produced during the radioactive decay would shift the isotopic composition of the uranium towards higher concentrations of the lighter isotope. A major aim of our study was therefore concerned with whether this effect could indeed be observed, for a positive outcome might shed light on the time interval involved in the plutonium fractionation.

There are also other possible explanations for the high concentrations of Pu—either the radioactive decay of a long-lived precursor of ^{239}Pu or the presence of a naturally occurring neutron source could result in the increased amount of ^{239}Pu (refs 1, 2). Our present experiments could not differentiate between these different hypotheses.

Because the dust present in the air deposits rather large amounts of Pu as fallout from nuclear tests in the atmosphere, we were concerned about contamination in our experiments and so obtained our samples from the collection of the Field Museum of Natural History in Chicago where, except for the ash from Mt St Helens, all samples had been collected before 1930 and stored since then in the vaults. To reduce further the possibility of contamination, all samples were processed either in a clean laboratory in the Chemistry Division building of Argonne

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Table 1 Chemical composition of lava samples from Kilauea and ash from Katmai

Sample origin	Sample no.					
	Kilauea, Hawaii			Katmai, Alaska		
	G677	G678	G2565	G2571	G2581	G2350
SiO ₂	53.6	47.8	49.3	51.3	49.6	58.0
TiO ₂	2.37	2.10	2.62	2.65	2.67	0.47
Al ₂ O ₃	12.2	10.6	12.6	12.8	12.9	19.4
Fe ₂ O ₃	3.80	2.26	2.76	2.75	2.76	1.20
FeO	7.46	9.86	9.33	9.23	9.38	3.25
MnO	0.15	0.15	0.15	0.15	0.15	0.12
MgO	9.04	5.99	7.76	7.39	7.38	3.07
CaO	10.17	9.49	11.35	11.50	11.50	8.49
SrO	0.037	0.040	0.043	0.044	0.045	0.050
BaO	0.016	0.018	0.018	0.019	0.019	0.037
Na ₂ O	2.29	2.45	2.16	2.16	2.45	4.32
K ₂ O	0.36	0.48	0.48	0.48	0.48	0.60

Tabulated values are weight %.

National Laboratory or in a clean laboratory in the Environmental Research Division.

A series of control samples was processed to determine the purity of the reagents and demonstrated that uranium was almost absent. The uranium levels in these controls were ~1 ng, <0.1% of the uranium in the actual samples. In addition, the isotopic ratio of the uranium in the control samples was found to be normal. All samples indicating any degree of enrichment in ²³⁵U were processed at least twice in both laboratories as an additional safeguard against contamination.

Complete chemical analyses and X-ray powder diffraction patterns were obtained for the Hawaiian lavas and the ash from Katmai, as the unusual ²³⁵U/²³⁸U ratios are associated with these materials. The chemical compositions are listed in Table 1. Major constituents identified by X rays were feldspar and pyroxene in the Hawaiian lavas, with the occasional occurrence of olivine in lesser amounts. The only phase identified in the ash from Katmai was feldspar. The composition data show the Hawaiian material to be tholeiitic, as expected for the region⁴. However, the trace element compositions, notably the level of uranium, suggest that some crustal material found its way into the basaltic lava. The composition of the ash from Katmai is fairly representative of andesitic basalts of the east Pacific rise. Here too, the trace element composition indicates an admixture of some crustal material.

The samples could be completely dissolved by leaching with HCl, followed by treatment of the residue with a mixture of HNO₃ and HF; the HCl solution was evaporated, redissolved in HNO₃ and added to the residue solution.

Pu and Th were extracted by passing the sample solution, 8 M in HNO₃, through a column containing Dowex I anion-exchange resin whose effluent contained the uranium fraction. This was evaporated to dryness, taken up in 9 M HCl then passed through a second anion-exchange column for separation of uranium from titanium. The uranium, together with iron, was eluted from the column with 1 M HCl. The iron was removed by multiple extraction of the uranium into diethyl ether from a

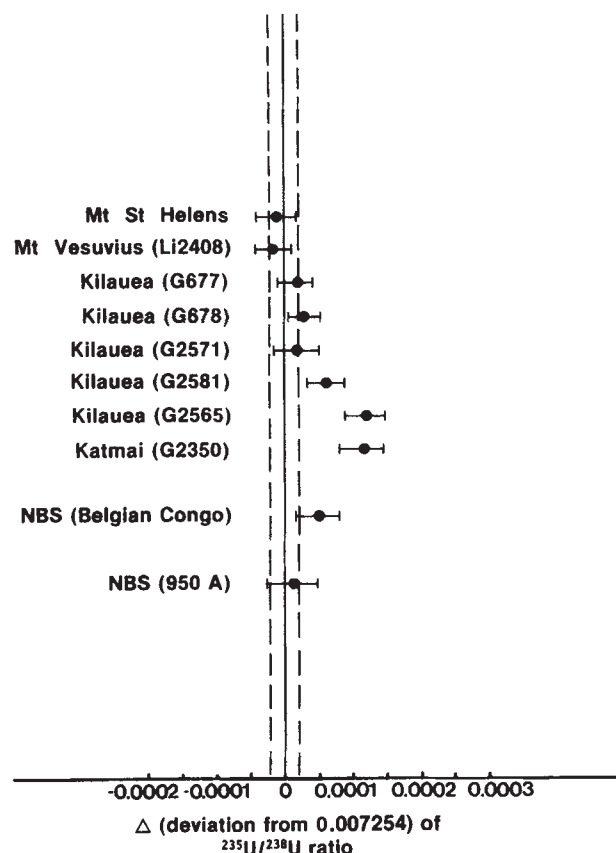


Fig. 1 Deviations from the mean value (0.007254) of the ²³⁵U/²³⁸U ratio obtained for the samples listed in Table 1. The solid vertical line represents the mean value of the report of the Subcommittee for the Assessment of Isotopic Abundances of the International Commission of Atomic Weights and Isotopic Abundances⁵, the dashed lines represent the limits of their variations.

1 M HNO₃ solution saturated with NH₄NO₃. Finally, the uranium was re-extracted with water and this solution evaporated to dryness after addition of a few drops of HClO₄ to destroy organic impurities. The plutonium was obtained from the first anion-exchange column by elution with 0.1 M HCl after the thorium had been removed with concentrated HCl. Pu was electrodeposited onto counting plates for assay.

Uranium-isotope ratios were measured on an automated triple-filament thermal-ionization source mass spectrometer equipped with a Faraday-cup collector. Corrections for fractionation were made by addition of a double spike of ²³⁴U and ²³⁶U, which had been previously calibrated with National Bureau of Standards U-500 isotopic standard. At least one unspiked sample from each source was analysed to determine minor isotope composition. This technique permits intercomparison of ²³⁵U/²³⁸U ratios at the natural abundance level to better than ±0.05% on 1-μg samples.

Table 2 Uranium and plutonium content of selected volcanic materials

Sample no.	Source	Year of eruption	Type of material	Uranium concentration (μg per g)	Atomic ratio	
					10 ³ ²³⁵ U/ ²³⁸ U	Pu/U
	St Helens	1980	Ash	1.3	7.253 ± 0.003	(2.7 ± 0.4) × 10 ⁻⁹
Li2408	Vesuvius	1906	Ash	3.4	7.252 ± 0.003	(9.5 ± 4.0) × 10 ⁻¹¹
G677	Kilauea	1890	Lava	0.51	7.256 ± 0.003	(6.3 ± 3.0) × 10 ⁻¹¹
G678	Kilauea	1890	Lava	0.5	7.257 ± 0.002	(1.3 ± 1.3) × 10 ⁻¹⁰
G2565	Kilauea	1926	Lava	0.55	7.267 ± 0.003	(1.2 ± 0.6) × 10 ⁻¹⁰
G2571	Kilauea	1926	Lava	0.55	7.256 ± 0.003	(1.5 ± 0.6) × 10 ⁻¹⁰
G2581	Kilauea	1926	Lava	0.52	7.260 ± 0.003	(6.2 ± 6.2) × 10 ⁻¹⁰
G2350	Katmai	1912	Ash	0.82	7.267 ± 0.003	(1.6 ± 0.4) × 10 ⁻⁹

Our results obtained on samples of volcanic ash and lava from Mts St Helens, Vesuvius, Kilauea and Katmai are summarized in Table 2. All of the material, with the exception of ash from Mt St Helens, was collected before 1942 and was kept in closed containers. The plutonium content in these old samples was consistently found to be quite low. Contrary to the findings of Meier *et al.*¹, who report Pu/U ratios of $\sim 10^{-7}$ in samples from Kilauea, we find the ratio to be $\sim 10^{-10}$ or less, that is, only one order of magnitude more than the value observed¹ for uranium minerals. The reason for this discrepancy may lie in the natural inhomogeneity of the volcanic rocks or in lower levels of radioactive fallout contamination in the Field Museum material. We note that the use of α -spectroscopy for the measurement of Pu content does not differentiate between ^{239}Pu and ^{240}Pu . The isotopic ratios of the Pu could be determined by mass spectrometry if larger samples were available.

An unusual feature is evident in the isotopic composition of the uranium in our samples. Whereas most of the analyses yield $^{235}\text{U}/^{238}\text{U}$ ratios close to the normally observed value of 7.254×10^{-3} , there are three instances of a statistically significant enhancement of the $^{235}\text{U}/^{238}\text{U}$ ratio in samples from Katmai (sample G2350) and from Kilauea (samples G2565 and G2581) for which multiple independent analyses were performed to minimize the error through contamination. The increase of the ratio by 0.18% to 0.007267 is well outside the error limit of the mass spectrometric uranium determination.

A previously reported survey of results obtained from a large number of uranium ores and minerals indicates a mean ratio of

$^{235}\text{U}/^{238}\text{U}$ of 0.007253, with a variation of ± 0.000003 ⁵. The excess found in our experiments for the two most enriched samples is about five times this variation (Fig. 1).

Of the samples showing an increased $^{235}\text{U}/^{238}\text{U}$, only the one from Katmai has an observable amount of Pu. The sample of the ash taken from the 1980 eruption of Mt St Helens has an observable amount of Pu, but no enrichment of ^{235}U . It is obvious that there is no simple relation between the observed concentrations of Pu and ^{235}U . This may be caused by fractionation of Pu and U during the process, or it may have been that the time interval for decay of the ^{239}Pu has been too short in the case of Mt St Helens and too long in the case of Kilauea.

Although our data should not be taken as being conclusive, we have not been able to confirm the high plutonium concentrations in volcanic materials reported in the literature. A clearer picture may be formed only on the basis of a more comprehensive sampling of other volcanic rocks.

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Methylgermanium in natural waters

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Biological methylation of metallic elements¹ and the occurrence and cycling of organometals in the environment have been investigated in Japan during the 1950s and 1960s, where the ingestion of fish and shellfish contaminated with methylmercury compounds caused mercury poisoning^{2,3}. Biomethylation ability has been demonstrated in bacteria, fungi and algae for As, Cd, Hg, Pb, Se, Sn, Te and Ti⁴⁻⁷. Naturally-occurring methylated species have been reported in estuaries and seawater for Sb⁸, As⁹, Ge¹⁰⁻¹³ and Sn¹⁴, although recent evidence suggests that some reports of methyltin species result from interferences by Ge and volatile sulphur compounds¹⁵. With the exception of Sb⁸, As^{9,12,16} and Ge^{11,12}, there are no consistent estuarine or oceanic profiles for methylmetal compounds in the literature from which to judge their biogeochemical behaviour. Early investigations¹⁷⁻¹⁹ reported that methylgermanium species do not exist in the aquatic environment. Subsequently, we identified monomethylgermanium (MMGe) and dimethylgermanium (DMGe) in estuaries^{10,12}, the Baltic Sea¹¹ and the Bering and Sargasso seas¹³. We report here recent measurements of methylgermanium compounds in river water, estuaries, seawater and anoxic basins.

Sampling locations are listed in Table 1. Methylgermanium was determined by hydride generation and graphite furnace atomic absorption spectrophotometry using 200-ml samples¹³. Analytical precision is approximately $\pm 1\%$ in the 300 pM range. Detection limits are 7 pM for MMGe, 10 pM for DMGe and 4 pM for TMGe (trimethylgermanium). We have not detected TMGe in natural waters.

Methylgermanium species were found to be conservative in a wide variety of estuaries, including pristine (Suwannee R. estuary), anthropogenically contaminated (Changjiang R. estuary; Delaware Bay; Tamar R. estuary), low productivity

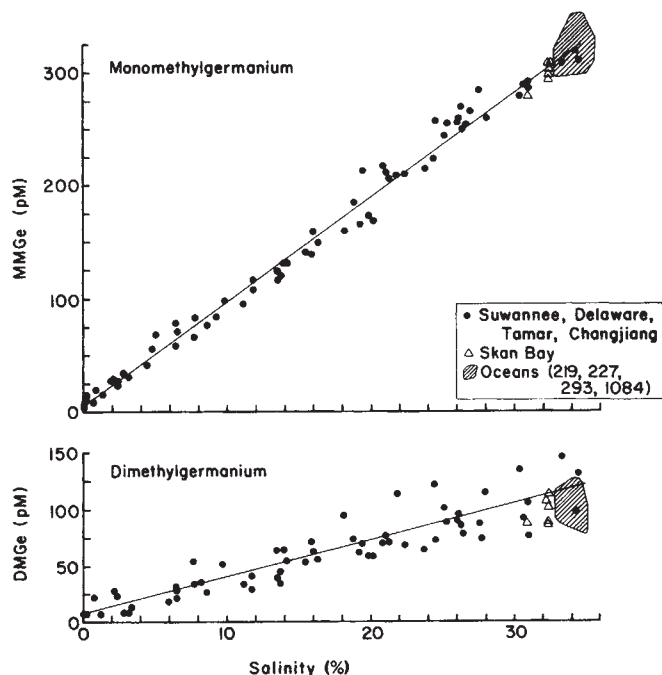


Fig. 1 Monomethylgermanium (MMGe) and dimethylgermanium (DMGe) concentrations versus salinity in estuaries, oceans and Skan Bay. The 35‰ seawater endmembers based on the estuarine data are 330 ± 15 pM for MMGe and 120 ± 20 pM for DMGe, consistent with our oceanic data.

(Suwannee; Changjiang at $S < 20\%$) and high productivity (Delaware; Changjiang at $S > 20\%$). Delaware Bay is contaminated by industrial and municipal wastes, agricultural fertilizers and pesticides²⁰. The Tamar R. estuary contains high levels of As^{21,22} and Mn²³, which are leached from abandoned mining operations in the Tamar R. drainage basin. Elevated levels of inorganic Ge (Ge_i) in the Tamar R. and estuary are probably derived from coal combustion²⁴. Similar high concentrations of

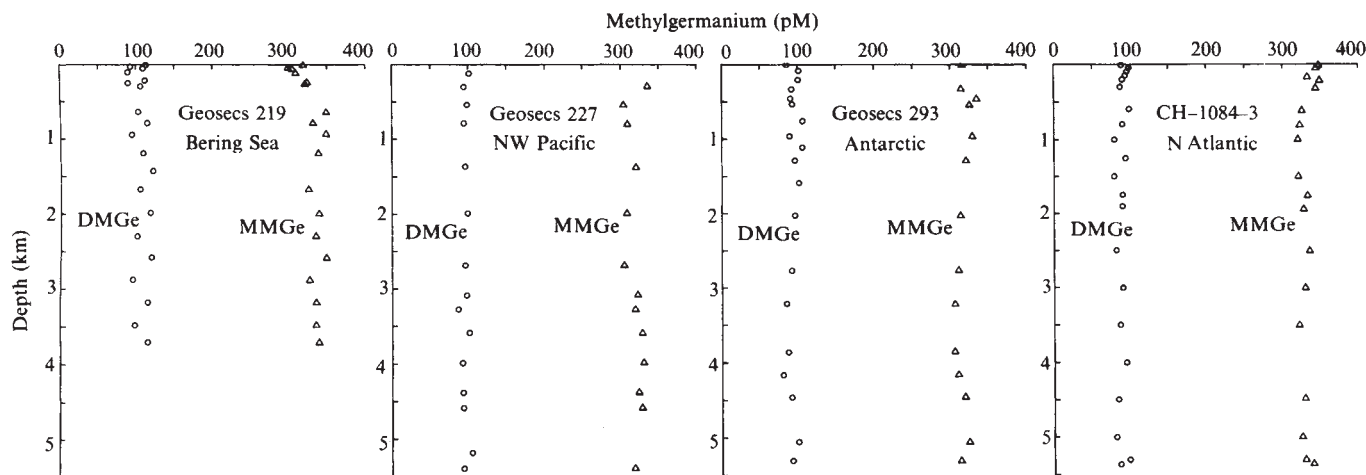


Fig. 2 Vertical profiles of monomethylgermanium (MMGe) and dimethylgermanium (DMGe) at four ocean stations.

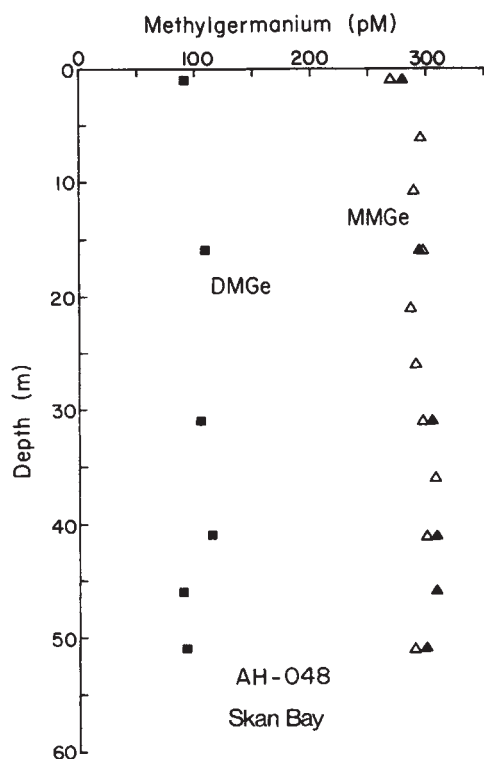


Fig. 3 Vertical profiles of monomethylgermanium (MMGe) and dimethylgermanium (DMGe) in Skan Bay. Open symbols, samples stored in polyethylene; solid symbols, those in sealed glass.

Ge_i in the Tejo estuary¹⁴ and in the Baltic Sea¹¹ are the result of pyrite-ore roasting and coal combustion, respectively. The Changjiang R. estuary has a high sediment load which suppresses primary productivity at salinities of <20‰, making it an ideal site for the study of non-biological processes²⁵. The Suwannee R., originating in the Okefenokee Swamp and flowing through a sparsely populated area of Florida, is essentially unaffected by human activity.

Plots of MMGe and DMGe concentration against salinity for axial sections of these estuaries (Fig. 1) are linear in all cases, suggesting that these species behave conservatively in the estuarine environment (that is, there is no production or destruction^{26,28}). The 35‰ seawater endmembers based on the estuarine

Table 1 Locations of sampling sites

Hydrographic ocean stations			
Geosecs 219	53°06' N	177°17' W	Bering Sea (N. Pacific)
Geosecs 227	25°00' N	170°05' E	NW Pacific
Geosecs 293	52°40' S	178°05' W	Antarctic (S. Pacific)
CH-1084-3	30°04' N	71°09' W	Sargasso Sea (N. Atlantic)
Estuaries			
Changjiang estuary (Yangtze)			Republic of China
Delaware Bay			Delaware
Suwannee R. estuary			Florida
Tamar R. estuary			SW England
Other			
Skan Bay (anoxic basin)			Unalaska Is., Alaska
Rainwater			
September 1983–July 1984			Tallahassee, Florida
(35 samples)			
5 August 1983 (1 sample)			Bering Sea (N. Pacific)
(53°11' N 177°17' W)			
24 July 1983 (1 sample)			New Zealand
7–16 November 1983			Caribbean Sea
(6 samples)			

Seawater samples were acidified to pH 2 with hydrochloric acid (CH-1084-3 was unacidified). Estuarine samples were filtered immediately (0.4 µm) and stored unacidified (Tamar was acidified). Seawater samples were analysed over an interval of two months (CH-1084-3) to more than 11 yr (Geosecs) following sample collection. Estuarine samples were analysed with intervals from two weeks (Suwannee) to 2½ yr (Changjiang) from collection. One profile in the Bering Sea (Alpha Helix-048-4/7), collected at the same location as Geosecs 219, was stored in glass bottles and yielded the same methylgermanium concentrations as those determined from Geosecs 219 samples (B. L. Lewis, unpublished data). There do not appear to be any significant artefacts due to sampling or storage procedures. Rain water samples were collected with a polyethylene sampler, acidified and stored cold in polyethylene bottles. Analyses were conducted within one month of sample collection.

data are 330 ± 15 pM-MMGe and 120 ± 20 pM-DMGe. Conservative behaviour for methylgermanium in Delaware Bay and the Tamar indicates that there are no anthropogenic sources of MMGe and DMGe and in the Changjiang it suggests that the methylgermanium compounds are unaffected by adsorption, desorption and flocculation processes. There is no evidence of biological reactivity for methylgermanium in estuaries of high productivity. Thus, we conclude that the organogermanium species are efficiently rejected during diatom silica uptake and are not produced during biogenic opal formation and regeneration¹², in contrast to the behaviour of Ge_i, which is taken up by, and regenerated from, the opaline tests of diatoms as if it were a super-heavy stable isotope of silicon^{12,19,24}. In Delaware

Table 2 Recoveries of inorganic Ge after ultraviolet-irradiation/oxidation of methylgermanium

Sample	Initial content (ng-Ge)				Final content (ng-Ge)				Recoveries (as % Ge _i)		
	Ge _i	MMGe	DMGe	TMGe	Ge _i	MMGe	DMGe	TMGe	MMGe	DMGe	TMGe
Deionized water plus standards	0	1.75	0	0	1.74	0	0	0	99	—	—
	0	0	2.19	0	2.06	0	0	0	—	94	—
	0	0	0	1.45	1.48	0	0	0	—	—	102
Artificial seawater plus standards	0	1.75	0	0	0.69	1.08	0	0	39	—	—
	0	0	2.19	0	0.58	1.28	0.19	0	—	27 (85)	—
	0	0	0	1.45	0.29	1.00	0.19	0	—	—	20(90)
Geosecs-219 (Bering Sea 3,479 m)	1.14	2.62	0.82	0	1.11	2.64	0.87	0	~0	~0	—
Sargasso surface seawater	0	2.45	0.65	0	0	2.34	0.55	0	~0	~0	—
Surface Sargasso seawater plus standards	1.09	3.69	1.43	0.76	1.08	3.70	1.51	0.80	~0	~0	~0

Samples irradiated for 5 h using a 1,250 W medium-pressure Hg lamp²⁹. Zeros, concentrations below detection limits. Ge_i, inorganic germanium; MMGe, monomethylgermanium; DMGe, dimethylgermanium; TMGe, Trimethylgermanium. Values in parentheses are % of initial methylgermanium converted to all other species.

Bay and the Changjiang, Ge_i is removed almost completely because of the activity of siliceous organisms (B.L., unpublished data). Alkali digestions of plankton and opal samples have not revealed the presence of methylgermanium, suggesting that diatoms do not sequester large quantities of organogermanium compounds intracellularly or in their frustules.

Oceanic vertical profiles of MMGe and DMGe (Fig. 2) are consistent with these estuarine data. Concentrations of the methylgermanium species in seawater are 326 ± 14 pM for MMGe and 97 ± 9 for DMGe, close to the seawater endmember concentrations in Fig. 1. The methylgermanium species are conservative throughout the water column, displaying no surface-to-deep water variation in concentration which cannot be explained by variations in salinity. There is no evidence of biological reactivity of methylgermanium in seawater and no apparent interocean differences. By contrast, concentrations of Ge_i at these same stations range from almost zero at the surface up to 180 pM in the bottom waters, mimicking vertical silica profiles. Thus, Ge_i and silica have virtually identical behaviour ($\text{Ge}_i/\text{Si} \sim 0.7 \times 10^{-6}$), reflecting uptake into, and dissolution from, the frustules of siliceous organisms²⁴. Methylgermanium profiles at these stations are essentially uniform, indicating that they are not involved in the Si biogeochemical cycle. Methylgermanium species in Skan Bay (Fig. 3) and the Baltic Sea¹¹ exhibit conservative behaviour, suggesting that they are neither produced nor consumed during anoxic diagenesis.

In a total of 35 rain-water samples collected at Tallahassee, Florida, from September 1983 to July 1984, DMGe was detected in five samples (DMGe < 15 pM) and MMGe in two samples (MMGe < 7 pM). Hambrick *et al.*¹³ reported DMGe and TMGe in stored rainwater samples ranging in concentration from 14 to 69 pM for DMGe and 3 to 14 pM for TMGe. We now consider the values reported for all of these samples to be artefacts resulting from contamination in the laboratory or from biomethylation of naturally-occurring Ge_i in rainwater by fungi growing in the sample storage bottles. Samples of marine rain collected carefully in New Zealand, the Bering Sea and the Caribbean Sea contained no detectable methylgermanium.

Methylgermanium species in seawater are stable to oxidation by persulphate¹³ and ultraviolet radiation (Table 2). In an initial ultraviolet radiation experiment, methylgermanium species in deionized water, artificial seawater and natural seawater were oxidized to Ge_i with recoveries of ~100%, 30% and <2%, respectively. TMGe and DMGe in artificial seawater are broken down preferentially to MMGe, suggesting that the conversion of MMGe to Ge_i is a rate-limiting step. This may account for the high concentration of MMGe in seawater.

Analysis of a large volume sample (1,200 ml) of Ochlockonee R. (Florida) water, by multiple borohydride reduction of 400 ml

volumes, allowed us to determine an upper limit for the concentrations of methylgermanium species in river water. The observed concentrations were 3 ± 2 pM (MMGe) and 7 ± 2 (DMGe), consistent with river endmember concentrations estimated from the estuarine profiles in Fig. 1 (MMGe ~4 pM; DMGe ~9 pM).

The sources and sinks of methylgermanium are as yet unknown. If terrestrial production, possibly biomethylation, of methylgermanium is its primary source of supply via rivers to the oceans, then its residence time in the ocean is several million years. This is consistent with our observations that methylgermanium is very stable in seawater and behaves conservatively.

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Ages of tuff beds at East African early hominid sites and sediments in the Gulf of Aden

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The early hominids of East Africa were dated by determining the ages of tuff beds at the sites. Despite much research using palaeomagnetic and K/Ar-dating techniques, some of those ages are still controversial^{1,2}. To obtain independent age estimates for these tephra layers, we have examined cores from DSDP Sites 231 and 232 in the Gulf of Aden (Fig. 1a) which consist mainly of calcareous nannofossil ooze, but also contain rare tephra horizons³ dated by interpolation from the established nannofossil stratigraphy (Fig. 1b). Chemical analysis confirms that the identity and sequence of these horizons is the same as that at the East African sites. We conclude that the age of the Tulu Bor Tuff is <3.4 Myr and hence that the Hadar hominid specimens are also <~3.4 Myr old.

The initial late Cenozoic calcareous nannofossil biostratigraphy of sites 231 and 232 (ref. 4) was based largely on the zonation of Bukry⁵ and recently has been more precisely dated^{6,7}. We recognized the following last-appearance datums (LADs) of calcareous nannofossil zones and assigned the following ages in Hole 231: *Calcidiscus tropicus* (Kamptner)⁷, usually referred to as *C. mcintyeri* Bukry and Bramlette, 1.42 Myr; *Discoaster brouweri* Tan, 1.88 Myr; *D. pentaradiatus* Tan, 2.35 Myr; *D. tamalis* Kamptner, 2.65 Myr; and *Reticulofenestra pseudumbilica* Gartner, 3.56 Myr. *D. tamalis*, although quite rare, has a well-defined range and was used also by Backman and Shackleton⁶, but not by Gartner *et al.*⁷. *D. asymmetricus* Gartner was not used because it has a discontinuous range and different authorities disagree on the last occurrence of this species^{6,7}. The LAD of *R. pseudumbilica* is well constrained because only specimens of *Reticulofenestra* with large diameters of more than 6 μm were included in this species; smaller specimens of *Reticulofenestra* (*R. haqii* Backman, *R. minuta* Roth, *R. minutula* Gartner and the related *Crenalithus productellus* Bukry) all occur above the LAD of *R. pseudumbilica*. The first-appearance datum (FAD) of *Ceratolithus acutus* Gartner and Bukry is 5.23 Myr.

Six tephra layers were sampled in cores of Hole 231 and two in cores of Hole 232. Glass shards, separated from samples of these tephra layers, were analysed by an electron microprobe using methods described previously⁸ and compared with samples (analysed concurrently) of tephra layers from the Koobi Fora region of the Turkana Basin in northern Kenya using the similarity coefficient (*SC*)⁹ for five elements (Table 1). MgO and MnO were not used in the numerical comparison because they occur in low and variable concentrations in the glass relative to precisions attainable with the electron microprobe. Na₂O and K₂O were not used because, in the Turkana Basin, their concentrations show systematic variations depending on the geographic locations of the tephra layers (T. E. Cerling, F.H.B. and J. R. Bowman, in preparation) even though such layers can be correlated stratigraphically using independent evidence (F.H.B., unpublished data). We attribute these variations to differences in degree of post-depositional alteration. We did not analyse for minor and trace elements because insufficient material was available.

The lowest vitric tephra layer in Hole 231 (sample 22-1; 189 m; Fig. 1b), is ~30 cm thick with pyroclasts (maximum dimension (m.d.) = 300 μm) consisting mainly of clear colourless angular platy bubble-wall shards and also some subordinate bubble-wall-junction and tubular pumice shards. This matches the composition of the Moiti Tuff of the Turkana Basin¹⁰ (*SC* = 0.96, where 1.00 represents 'identity') for which a maximum age of

4.10 $\pm$ 0.07 Myr has been determined¹¹, which supports our estimate of 3.9 Myr, based on calcareous nannofossil zones in Hole 231 and interpolation between these zones assuming a constant sedimentation rate (Fig. 2).

The tephra layer at 180 m in Hole 231 (sample 21-2), ~7 cm thick and exhibiting similar pyroclasts (m.d. = 295 μm) to those of sample 22-1, correlates with tephra layer K81-485 (F.H.B., unpublished data) (*SC* = 0.96) which lies between the Lokochot and Moiti Tuffs in the Turkana area. For this layer, an age of 3.7 Myr can be assigned on the basis of biostratigraphic zonation in Hole 231 (Fig. 2).

At 170 m in Hole 231 (sample 20-2), a tephra layer ~10 cm thick, and composed mainly of clear bubble-wall shards (m.d. = 400 μm) with subordinate bubble-wall-junction and tubular pumice shards having long (90- μm) large cylindrical vesicles, matches well the high-iron glass shards of the Lokochot Tuff of the Koobi Fora Formation (80-295; *SC* = 0.97). (Glass shards of the Lokochot Tuff (Tuff A of the Shungura Formation¹⁰) have a bimodal composition with respect to iron (F.H.B., unpublished data), the iron-rich variety having 4.5%, and the iron-deficient variety ~3.2%, Fe₂O₃.) It also correlates well with the tephra layer at 165.3 m in Hole 232A (sample 1-5, *SC* = 0.97; Fig. 1a, Table 1) which has smaller shards (m.d. = 240 μm) of the same type; 1.3 m above at 164-m depth (sample 1-4) in Hole 232A, the tephra layer also correlates very well with the average composition of the Lokochot Tuff (80-295; *SC* = 0.98). Assuming a constant sedimentation rate at site 232, we can estimate an age difference of ~50 kyr between the tephra layers at 165.3 and 164 m in this hole and, on the basis of biostratigraphic zonation, we can assign an age of ~3.5-3.6 Myr to sample 20-2 in Hole 231 and samples 1-4 and 1-5 in Hole 232 (Fig. 2).

The tephra layer at 161 m in Hole 231 (sample 19-2) contains abundant clear colourless angular platy bubble-wall shards (m.d. = 380 μm) and subordinate bubble-wall-junction shards with straight 'ribs', and some tubular pumice shards having elongated rod-shaped vesicles. The glass shards of this tephra layer are identical in composition to the β -Tulu Bor Tuff of the Koobi Fora Formation (*SC* = 0.98; Table 1), Tuff B- β of the Shungura Formation and the Sidi Hakoma Tuff of the Hadar Formation (see also ref. 1). This layer is situated in the *D. tamalis* zone and is assigned an age of 3.2 Myr by interpolation between the calcareous nannofossil-datum planes (Fig. 2).

The tephra layer at 119 m in Hole 231 (sample 14-5) is 1 cm thick, and contains shards (m.d. = 215 μm) similar to those of the lower layers. The age of this layer is ~2.2 Myr based on biostratigraphy of the core, but, for this ash, there is no clear correlation with any from East Africa.

Finally, a 2-mm-thick tephra layer at 80-m depth in Hole 231 (sample 10-5) is estimated to be ~1.5 Myr, somewhat similar in age and composition to the Chari Tuff of the Koobi Fora Formation (ref. 11 and F.H.B., unpublished data), but older and higher in iron content (Table 1).

Volcanic glass of the four lower tephra layers in DSDP Hole 231 are chemically identical, within the limits of the electron-microprobe technique for the five elements used in our comparison, to their four counterparts in the northern Turkana basin of East Africa: the Moiti, K81-485, Lokochot and the β -Tulu Bor Tuffs. Furthermore, the sequence of chemical types of the tephra layers is the same within stratigraphic sections at both localities. Lastly, independent numerical age control in each of the two correlated sequences indicates that they are coeval. Thus, the Moiti Tuff, the lowermost tephra layer in the Turkana basin, has a maximum age of 4.1 Myr¹¹, and our estimate of the age of its correlative in sediments of the Gulf of Aden, also the lowermost silicic tephra layer in the core, is 3.9 Myr, based on calcareous nannofossil chronology. The Tulu Bor Tuff has not been dated directly in the Turkana basin, but underlies the Toroto Tuff (dated at 3.3 Myr¹¹) with little stratigraphic separation; its age must therefore be very close to 3.3 Myr. As our estimate for the correlative of this tuff in the Gulf of Aden is 3.2 Myr, based on the calcareous nannofossil chronology, we consider the correlations to be well substantiated.

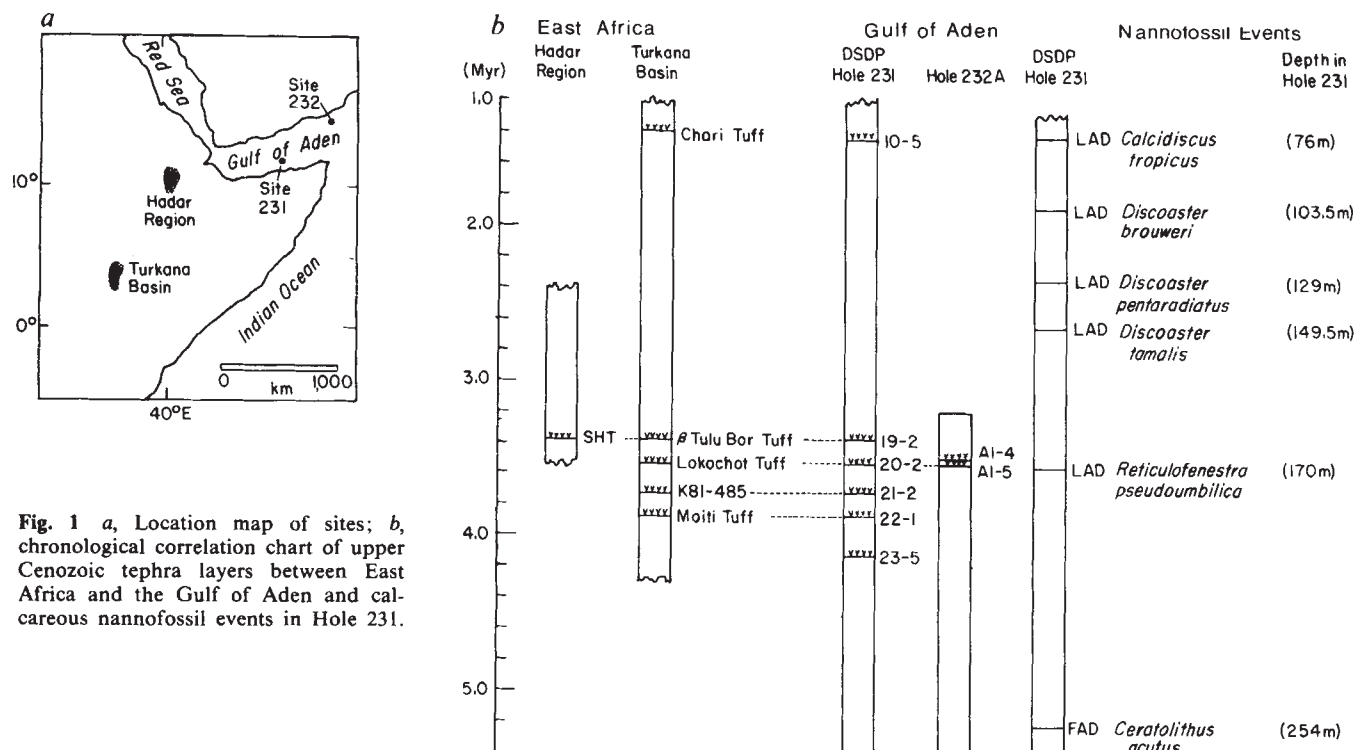


Fig. 1 a, Location map of sites; b, chronological correlation chart of upper Cenozoic tephra layers between East Africa and the Gulf of Aden and calcareous nannofossil events in Hole 231.

Table 1 Electron-probe analysis of volcanic glass shards separated from tephra layers of deep-sea cores in the Gulf of Aden (DSDP holes 231 and 232) and in the Turkana Basin, Kenya

Sample number, standard deviation, and number of shards analysed	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	MnO	CaO	TiO ₂	Na ₂ O	K ₂ O	T ₀
Tephra layer at 80 m in DSDP hole 231										
(1) 10-5 (69-71 cm) ±σ (20 shards)	74.57 0.55	12.03 0.18	3.35 0.10	0.00 —	0.09 0.01	0.25 0.01	0.16 0.02	5.18 0.15	4.37 0.09	92.60
Chari Tuff, Turkana Basin										
(2) 77-23 ±σ (20 shards)	76.92 0.89	10.94 0.25	2.76 0.08	0.00 —	0.09 0.02	0.18 0.01	0.19 0.04	4.90 0.28	4.02 0.45	94.10
Tephra layer at 90 m in DSDP hole 231										
(3) 14-5 (109-111 cm) ±σ (15 shards)	74.13 2.45	10.44 1.64	4.97 0.90	0.12 0.06	0.23 0.04	0.21 0.10	0.37 0.09	4.89 0.50	4.64 0.34	92.76
Tephra layer at 160 m in DSDP 231 (4) and β-Tulu Bor Tuff, Turkana Basin (5)										
(4) 19-2 (33-34 cm) ±σ (30 shards)	76.43 0.60	12.50 0.23	1.57 0.06	0.05 0.01	0.05 0.01	0.31 0.06	0.14 0.01	3.81 0.17	5.14 0.14	94.31
(5) K82-869 ±σ (16 shards)	76.97 1.56	12.77 0.33	1.60 0.11	0.05 0.01	0.05 0.01	0.30 0.02	0.14 0.03	4.46 0.19	3.67 0.24	91.50
Tephra layer at 164 m in DSDP 232A (6), and Lokochot Tuff (bulk), Turkana Basin (7, 8)										
(6) Al-4 (42-45 cm) ±σ (14 shards)	75.73 0.66	10.61 0.57	3.97 0.18	0.01 <0.01	0.16 0.02	0.19 0.02	0.20 0.05	4.56 0.26	4.58 0.20	92.89
(7) 80-295 ±σ (14 shards)	77.41 0.90	10.73 0.46	3.76 0.72	0.02 <0.01	0.12 0.02	0.19 0.02	0.20 0.04	3.66 0.50	3.91 0.36	90.42
(8) 80-293 ±σ (15 shards)	77.32 0.92	10.81 0.59	3.78 0.67	0.03 0.01	0.13 0.02	0.20 0.01	0.19 0.04	3.48 0.75	4.05 0.89	92.23
Tephra layer at 170 m in DSDP 231 (9), tephra layer at 165.5 m in DSDP 232A (10, 11), and Lokochot Tuff (high iron), Turkana Basin (12)										
(9) 20-2 (25-28 cm) ±σ (15 shards)	75.65 0.66	10.62 0.35	4.06 0.35	0.00 —	0.16 0.02	0.19 0.02	0.23 0.03	4.38 0.29	4.71 0.24	92.48
(10) Al-5 (25-27 cm) ±σ (15 shards)	75.39 0.79	10.53 0.45	4.14 0.37	0.01 <0.01	0.16 0.02	0.19 0.02	0.24 0.04	4.69 0.30	4.66 0.22	92.68
(11) Al-5 (28-29 cm) ±σ (15 shards)	75.59 0.56	10.57 0.42	4.03 0.55	0.01 <0.01	0.16 0.01	0.19 0.03	0.23 0.04	4.55 0.26	4.69 0.24	92.38
(12) 80-295 ±σ (5 shards)	77.50 0.58	10.52 0.20	4.47 0.02	0.02 <0.01	0.15 0.02	0.19 0.01	0.22 0.04	3.22 0.97	3.70 0.66	90.25
Tephra layer at 180 m in DSDP 231 (13), and Tuff K81-485, Turkana Basin (14)										
(13) 21-2 (15-17 cm) ±σ (15 shards)	76.87 0.68	11.44 0.24	2.42 0.06	0.01 <0.01	0.07 0.01	0.18 0.01	0.13 0.02	4.09 0.16	4.79 0.17	93.20
(14) K81-485 ±σ (17 shards)	77.83 0.64	11.41 0.25	2.50 0.07	0.03 0.01	0.07 0.01	0.20 0.01	0.12 0.02	3.64 0.30	4.20 0.25	92.25
Tephra layer at 189 m in DSDP 231 (15), and Moiti Tuff, Turkana Basin (16)										
(15) 22-1 (82-85 cm) ±σ (15 shards)	76.68 0.54	11.28 0.25	2.73 0.07	0.00 —	0.08 0.01	0.21 0.01	0.19 0.03	4.24 0.17	4.58 0.16	92.94
(16) 81-602 ±σ (16 shards)	76.97 0.77	11.22 0.36	2.80 0.06	0.01 <0.01	0.10 0.01	0.22 0.01	0.17 0.03	4.11 0.36	4.40 0.26	93.72

Concentrations in oxide-wt%, recalculated to 100% on a fluid-free basis. Original totals (T_0) are given to indicate degree of hydration of glass, and for recalculating oxides to original analysed values.

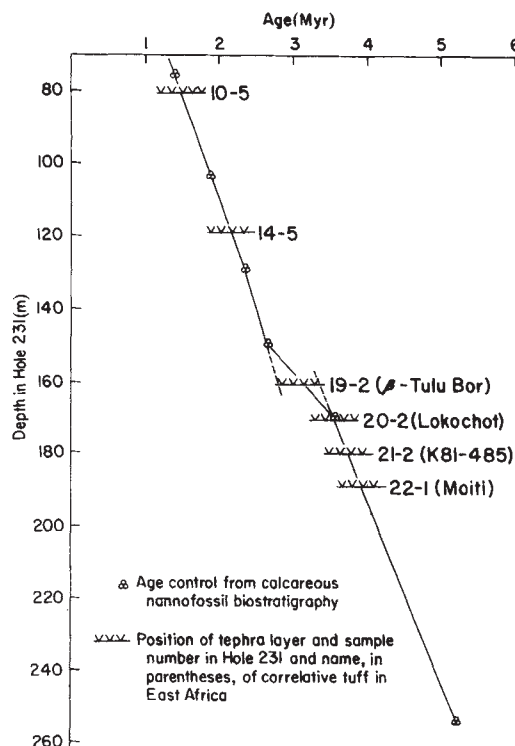


Fig. 2 Depth in Hole 231 plotted against ages estimated from calcareous nannofossil biostratigraphy.

One of the more important results gained from this study is independent confirmation of the age of the β -Tulu Bor Tuff of the Turkana Basin, which has been recently correlated with the Sidi Hakoma Tuff (SHT) of the Hadar region of Ethiopia¹. This has significant implications for the ages of hominid specimens in the Hadar Formation. Objections have been raised both to the correlation of these tuffs and to the chronology suggested for the Hadar Formation². But an objection to the former, namely, that the Tulu Bor Tuff had not been found between the Hadar region and the Turkana Basin, has been weakened greatly with identification of this ash in the Gulf of Aden. If the Tulu Bor Tuff occurs both in the Gulf of Aden and in the Turkana Basin, it should also occur in the Hadar region lying between these two areas (Fig. 1a). A principal objection to the chronology proposed for the Hadar region was that that of the Turkana Basin was also somewhat tentative. Although this has been rectified by very careful work¹¹, it was still not certain, until the present study, that the Tulu Bor Tuff was <3.4 Myr in age. There were two possible chronological slots to which the Tulu Bor Tuff could have been assigned, given that it is older than 3.3 Myr¹ and of normal polarity: 3.3–3.4 Myr and 3.8–3.9 Myr, the bottom of the Gauss Normal Chron and the Cochiti Normal Subchron (of the Gilbert Reversed Chron) respectively. Because the Tulu Bor Tuff falls within the *D. tamalis* zone (2.65–3.56 Myr) in core 19 of Hole 231, a clear choice can now be made in favour of the younger age.

We conclude that hominid specimens from Hadar are younger than ~3.3 Myr; with only a few exceptions, hominid specimens from the Turkana Basin are also younger than ~3.3 Myr. Our data also provide age estimates of certain undated tephra layers in East Africa: for the Lokochot Tuff, ~3.5–3.6 Myr, and for Tuff K81-485, ~3.7 Myr. Our findings suggest that in this region provincial vertebrate biostratigraphic zones can be correlated with those for global marine invertebrates, that marine biostratigraphic zones are linked to datable tephra on land, and that terrestrial fossil vertebrates can be placed in a climatic context by documenting secular oxygen-isotopic variations in deep-sea cores that are bracketed by widespread tephra layers.

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Loss of spatial phase relationships in extrafoveal vision

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Objects in peripheral vision are not simply blurred but lack quality of form¹. Assuming that the visual system performs a (patchwise) Fourier analysis of the retinal image (for review see ref. 2), it has been suggested that this disadvantage of peripheral vision may be due to the inability to encode properly spatial phase relationships^{3–5}. This is of great interest for neurological research as certain visual pathologies imply alterations of perceived form^{6,7}. Previous attempts at measuring phase sensitivities failed to distinguish between the detection of phase-related changes in contrast and phase coding in the visual system⁸. We separated these processing strategies by applying the iso-second-order texture paradigm of Julesz² to the discrimination of compound gratings. Our results, reported here, show that the energy detection properties of both foveal and peripheral vision are comparable, however, independently of scale, peripheral vision ignores the relative position of image components.

Phase coding, by definition, determines the relative positions of spatial frequency components of an image. This is not to be confused with encoding the position of points within an image, although the two descriptions are interrelated. The importance of phase in signals is obvious from the intelligibility of phase-only reconstructions for images, speech and crystallographic structures⁹. It is also clear that a disturbance of phase alters the appearance of an image in suprathreshold vision. Nevertheless, there is still confusion as to the exact role of phase in visual processing, mainly owing to the fact that phase modulation changes both the spectral composition of an image and its luminance profile. Thus, one can reliably study phase sensitivities only by having observers discriminate patterns that do not allow a simple contrast comparison; such patterns were used by Julesz² in his iso-second-order texture paradigm. This paradigm was used in the present study to investigate the respective roles of grating contrast detection and phase processing.

Changes in phase between sinusoidal grating components normally, but not always, change the spatial densities of compound patterns with identical power spectra. Consider a pair of compound gratings consisting of a fundamental and its third harmonic. If the phase of the gratings is varied around a mean of 0° (see also ref. 10), these gratings will be mirror images of each other: for example, in the case of a 120° phase separation, one grating has –60° phase angle and the other +60° (Fig. 1a). For intermediate values of mean phase, grating pairs differ in local contrast and, therefore, in their first-order statistics⁵; this is shown in Fig. 1b for a 120° phase separation and 90° mean

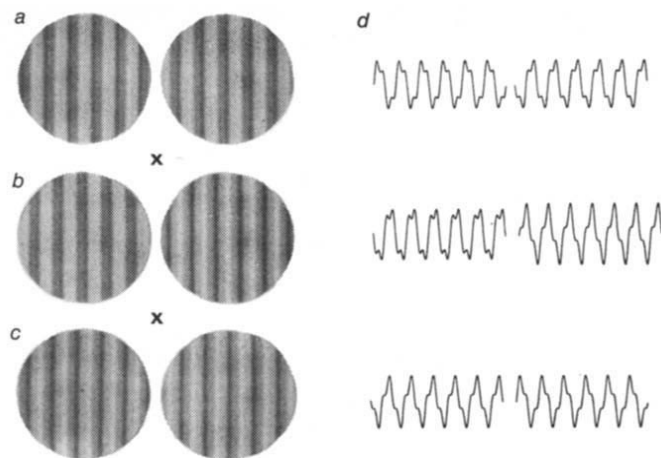


Fig. 1 Complex grating pairs with identical power spectra and the same phase separation (120°) between their first- and third-harmonic spatial frequency components. *a*, *c*, Mirror-image grating pairs; *b*, pair with different spatial contrast densities; *d*, corresponding luminance profiles. The stimulus pairs were generated by adding and subtracting a 60° phase angle to and from a given mean phase. In our experiments the patterns were presented sequentially at the same retinal location. This figure shows the gratings side-by-side to give an appreciation of the main finding: all patterns are readily distinguishable when looked at directly at a normal viewing distance. If either of the cross-targets is fixated, grating pairs *a* and *c* become indistinguishable, while pair *b* is still discriminated.

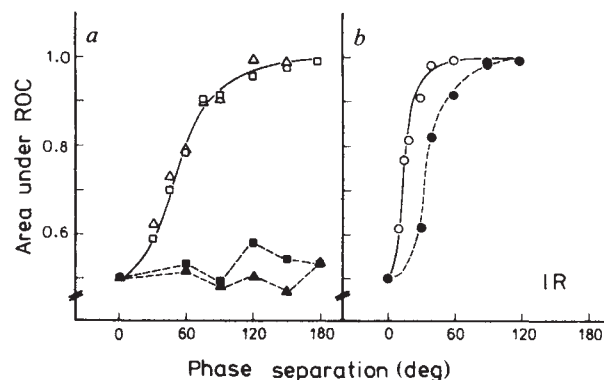


Fig. 2 Discrimination sensitivities for complex grating pairs as a function of their phase separation. *a*, 0° and 180° mean phase; *b*, 90° mean phase. Open symbols, fundamental spatial frequency of targets 3 cpd at 228 cm viewing distance, central observation. Solid symbols, fixation 2° away from the left edge of targets, viewing distance reduced to 140 cm. Observer I.R. Each data point was derived from an experimental run totalling about 100 noise trials (same images) and 50 signal trials (different images). The area under ROC was determined by least-squares solution (program RSCORE by L. O. Harvey Jr; for original version see ref. 20). Average s.d. was 0.034 for 0° and 180° mean phase, and 0.017 for 90° mean phase.

phase. At 180° mean phase the pairs again comprise mirror images (a 120° separation is shown in Fig. 1*c*). Such patterns have identical second-order statistics as they are transformed into each other by a rotation of 180° around their centres¹¹. Unlike grating pairs with intermediate phase, they only differ in the relative position of local regions having identical contrast.

The gratings were generated on an oscilloscope screen (HP 1304A, P4 phosphor) by a LSI 11/23 computer at a frame rate of 64 Hz. They were displayed through a circular window within a moderately illuminated screen (window diameter 3.2° , at a viewing distance of 228 cm). Average luminance of the targets was kept constant at 10 cd m^{-2} and the fundamental had a spatial frequency of 3 cycles per degree (cpd) at 228 cm distance; its contrast was 30%, while that of the third harmonic was fixed at 10%. The position of the gratings varied randomly between presentations. Subjects viewed the screen binocularly.

The observers were familiarized with a given pair of stimuli by unlimited exposure. Discrimination sensitivities were measured by using a signal-detection rating procedure¹². Trials consisted of presenting two patterns sequentially with a 47-ms exposure time and 700-ms inter-stimulus interval (ISI). The subjects were instructed to press one of six buttons, depending on whether they believed that the same (buttons 1–3) or different (buttons 4–6) patterns were being displayed. The area under the receiver-operating characteristic (ROC) was computed after 150 trials.

Figure 2 summarizes the results obtained with the experienced, emmetropic observer, I.R. (one of the authors). For central viewing conditions, no difference in discrimination sensitivities between grating pairs of 0° and 180° mean phase was found, while discrimination as such was clearly more difficult than for grating pairs of 90° mean phase. Rather dramatic differences were obtained in 2° off-axis viewing conditions, where discrimination sensitivities approached the chance level for both mirror-image and non-mirror-image pairs. By re-scaling the stimulus size according to cortical magnification¹³, that is, by reducing the viewing distance by a factor of 1.62 (to 140 cm), extrafoveal discrimination sensitivities were almost fully restored at 90° reference phase but not for mirror-image grating

pairs. This result has been confirmed by data for two other observers (G.B. and R.H.; see Fig. 3) who were ignorant as to the purpose of the experiment.

It is impossible to account for our data on the sole assumption of a special class of 'phase-sensitive devices' centred at 0° and 180° ¹⁴. Such a model may explain the relatively poor discrimination of grating pairs symmetrically spaced about the phase-selective mechanisms¹⁰, yet the non-discrimination of mirror-image gratings suggests that these mechanisms do not operate 2° away from the fixation point. The discrimination obtained in these conditions for grating pairs centred at 90° runs counter to such an assumption. Alternatively, it has been suggested that only one mechanism—the detection of differences in the contrast of local regions of the stimuli—need be proposed to explain the dependence of compound grating discrimination on the reference phase⁸. This model would predict the ability to discriminate between gratings differing in first-order statistics but it fails to explain how symmetries in mirror-image grating pairs are broken in foveal vision.

Thus, we are led to conclude that two different processes are involved in compound grating discrimination, neither of which can be related to narrow-band spatial frequency channels (see ref. 2) as the latter would not detect differences between gratings with identical power spectra. Our results are also incompatible with the notion that spatial phase is encoded *per se*. It is more likely that the two processes implicated so far take account of different aspects of image luminance profiles. Clearly, one process registers the positional relationships of image components; this process will be available only for foveal vision and may mediate the quality of form. A reasonable model of this process seems to be some form of linear matched filtering¹⁵ on the basis of even and odd symmetric receptive fields¹⁶, and the subsequent combination of such detector outputs. The other process registers contrast variability, but ignores positional information; a possible mechanism for this is energy detection as in hearing¹⁷, that is, spatial filtering with only one symmetry class of receptive fields. This process probably operates in both peripheral and foveal vision. Indeed, we also have evidence for separate contrast analysis and processing of positional information from experiments with centrally presented line stimuli¹⁸.

Finally, it is interesting to note that Julesz⁵ found iso-second-order textures that are globally indistinguishable although their elements can be distinguished by local scrutiny. In much the

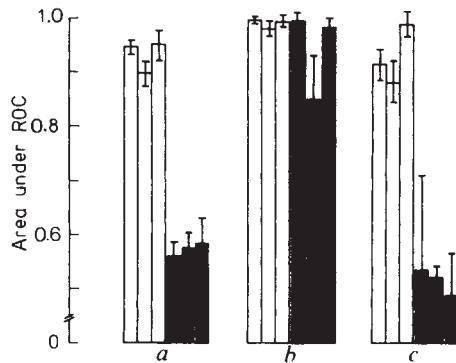


Fig. 3 Discrimination sensitivities for complex gratings with a phase separation of 120° . Mean phase in $a = 0^\circ$, $b = 90^\circ$ and $c = 180^\circ$. Open bars, central observation of 3 cpd targets at 228 cm viewing distance; solid bars, 2° off-axis observation of same targets at 140 cm viewing distance. Observers were G.B., R.H. and I.R. from left to right, respectively, for each group of three bars. Experimental conditions and data from observer I.R. as in Fig. 2.

same way, peripheral vision cannot break symmetries in iso-second-order grating pairs, the bars of which can be distinguished easily by focal attention. Furthermore, like textures in 'pre-attentive' vision, gratings differing in first-order statistics give rise to 'discrimination for trivial reasons'⁵. Julesz and co-workers even proposed that distinguishable iso-second-order textures are analysed by 'figure-like' processes, while their differences are ignored by 'ground-like' vision. Thus, it seems that the texton theory of Julesz and co-workers⁵ embraces the aspects of grating perception at issue here. However, there are clear differences in the experimental paradigms used by Julesz and ourselves. The texture pairs of Julesz⁵ were shown side-by-side with delays not exceeding 300 ms (the duration of short-term visual memory), whereas our grating data were obtained with sequential stimulation at the same retinal location and 700 ms ISI. The difference in spatial stimulus properties is not easily resolved, because two juxtaposed gratings would stimulate both foveal and extrafoveal regions; judging from an inspection of Fig. 1, however, this difference may not be crucial. Another difficulty is that our results imply a scale-invariant superiority of foveal vision, whereas Bergen and Julesz¹⁹ have shown how a disadvantage of peripheral vision can be overcome by shifting focal attention. Thus, the precise relationship between grating data and texton theory remains to be established.

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Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment

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Vertebrate rod photoreceptors hyperpolarize when illuminated, due to the closing of cation-selective channels in the plasma membrane. The mechanism controlling the opening and closing of these channels is still unclear, however. Both $3', 5'$ -cyclic GMP^{1,2} and Ca^{2+} ions³ have been proposed as intracellular messengers for coupling the light activation of the photopigment rhodopsin to channel activity and thus modulating light-sensitive conductance. We have now studied the effects of possible conductance modulators on excised 'inside-out' patches from the plasma membrane of the rod outer segment (ROS), and have found that cyclic GMP acting from the inner side of the membrane markedly increases the cationic conductance of such patches (EC_{50} 30 μM cyclic GMP) in a reversible manner, while Ca^{2+} is ineffective. The cyclic GMP-induced conductance increase occurs in the absence of nucleoside triphosphates and, hence, is not mediated by protein phosphorylation, but seems rather to result from a direct action of cyclic GMP on the membrane. The effect of cyclic GMP is highly specific; cyclic AMP and $2', 3'$ -cyclic GMP are completely ineffective when applied in millimolar concentrations. We were unable to recognize discrete current steps that might represent single-channel openings and closings modulated by cyclic GMP. Analysis of membrane current noise shows the elementary event to be 3 fA with 110 mM Na^+ on both sides of the membrane at a membrane potential of -30 mV. If the initial event is assumed to be the closure of a single cyclic GMP-sensitive channel, this value corresponds to a single-channel conductance of 100 fS. It seems probable that the cyclic GMP-sensitive conductance is responsible for the generation of the rod photoresponse *in vivo*.

Experiments were performed on the light-adapted retina of the frog *Rana temporaria*. Figure 1a shows the recording method. A retina treated with trypsin (10 mg ml^{-1} , 30 min, 20°C) was shaken gently in a small volume of saline and the solution containing detached ROSs and whole photoreceptor cells was then pipetted into a perfusion chamber. Under visual observation, the recording electrode was advanced to make contact with the lateral surface of the outer segment of a whole rod, or more often, an isolated ROS. Brief suction was applied to the pipette to produce a seal of 1-20 G Ω (ref. 4) and a patch of membrane was excised from the cell by a sharp shift of the pipette. This exposed the intracellular surface of the membrane to the bathing solution in the chamber while the extracellular surface was bathed with the solution contained in the pipette. The results reported below were obtained from membrane fragments lacking the high-conductive anion channels described previously⁵.

During these experiments, we obtained 136 patches sufficiently stable for the solution in the chamber to be changed several times. Of these, 70 responded to the application of cyclic GMP by an increase in conductance (see Fig. 1b), while the current fluctuated in a random manner without any recognizable steps which could represent single-channel activity (see below). The effect of cyclic GMP was completely reversible and could be observed several times in the same patch, with restoration to baseline when the cyclic GMP was washed out. The sensitivity of most patches to cyclic GMP remained constant for 10-15 min after the patch was isolated (during this time the solution in the chamber was changed five to eight times) before it began to decrease.

To our surprise, the cyclic GMP-dependent increase in the conductance of the patch occurred in the absence of nucleoside

Fig. 1 *a*, Recording method. The perfusion system had a 'dead volume' of ~ 2 ml and the flow rate was usually $3\text{--}5\text{ ml min}^{-1}$. If not otherwise stated, a standard Ringer solution containing (in mM) 90 NaCl, 10 KCl, 2 MgCl₂, 0.1 CaCl₂, 10 Na-phosphate pH 7.5 was used. The microelectrodes were always filled with this saline. The yield of vesicles in this low-calcium solution was $<20\%$ ⁵, so no precautions were taken against their formation. The pipette and bath contained Ag/AgCl pellet electrodes. Current flowing across the patch of membrane at the pipette tip was measured with a virtual ground circuit (*A*₁, operational amplifier with a $10\text{-G}\Omega$ feedback resistor). To improve time resolution, the recovery of high frequencies was used. The signal was low-pass-filtered (6-pole Bessel filter, cut-off frequencies within 500–10,000 Hz). The transmembrane voltage was controlled by applying a command voltage through a buffer operational amplifier, *A*₂. The voltage inside the measuring electrode was assumed to be zero in agreement with the electrophysiological convention that the extracellular space is at zero potential. *b*, Chart recording of the action of cyclic GMP (cGMP; Sigma) on an isolated patch of the ROS plasma membrane. The command voltage comprised 10-mV pulses (bottom trace). The upper trace represents the current flowing through the patch. Cyclic GMP was applied to the bathing solution.

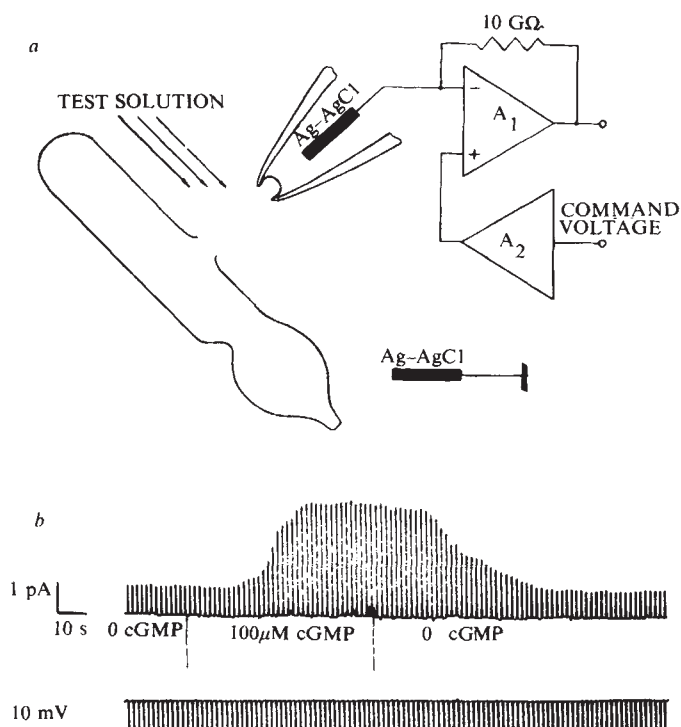
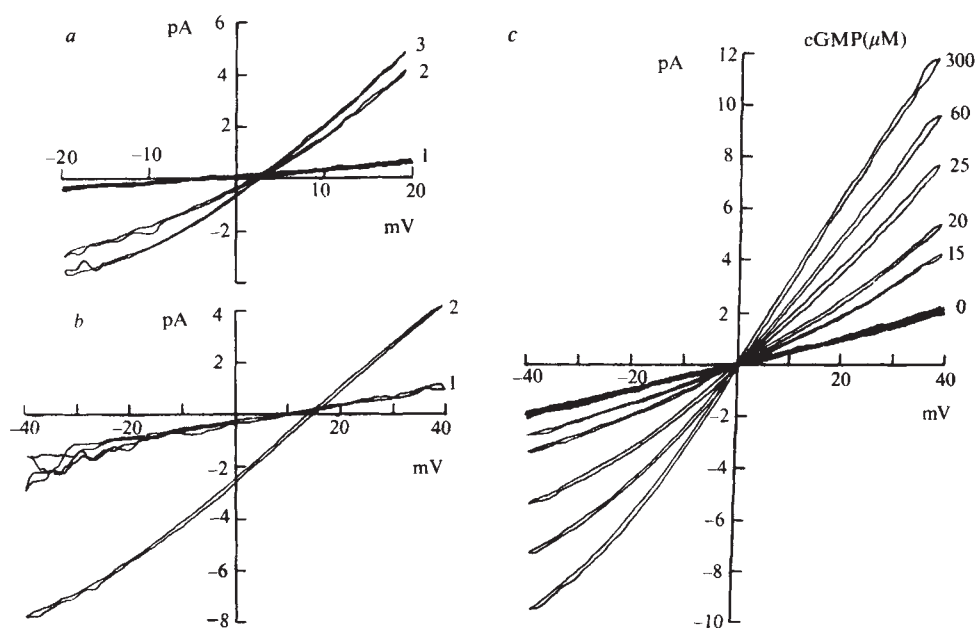


Fig. 2 Voltage-current relations of patches of the ROS plasma membrane. A saw-toothed voltage (5 mV s^{-1}) was applied to patches and the x-input of an x-y recorder. The output signal of the current-voltage converter was applied to the y-input of the recorder. The relations were recorded using one to three cycles of the saw-toothed voltage. The stability of the baseline was controlled after every application of cyclic GMP. *a*, Effect of Ca^{2+} and cyclic GMP on a patch of the ROS plasma membrane. Curve 1, 10 nM or 1 mM Ca^{2+} , no cyclic GMP; curve 2, 100 μM cyclic GMP, 10 nM Ca^{2+} ; curve 3, 100 μM cyclic GMP, 1 mM Ca^{2+} . The composition of the solution was (in mM): 100 KCl, 10 Na-phosphate, 2 MgCl₂, 1 CaCl₂ in high-calcium solution, 0.1 and 0.384 EGTA in the low-calcium one (the dissociation constants for Ca-EGTA complexes given in ref. 23 were used), pH 7.5. *b*, Voltage-current relation in a low-salt solution containing (mM): 45 NaCl, 5 KCl, 2 MgCl₂, 0.1 CaCl₂, 5 Na-phosphate, pH 7.5. Curve 1, no cyclic GMP; curve 2, 100 μM cyclic GMP. *c*, Family of voltage-current relations recorded at different cyclic GMP concentrations. Cyclic GMP was applied in standard Ringer. The relation at 500 μM cyclic GMP (not shown) was identical to that at 300 μM cyclic GMP.



triphosphates; the addition of 1 mM ATP and 1 mM GTP was without effect. It is generally considered that the effects of cyclic nucleotides are mediated by protein phosphorylation by specific kinases⁶. Analysis by TLC of the cyclic GMP preparation used shows it to contain $<1/10^6$ nucleoside di- and triphosphates, that is, a concentration of $<10^{-10}$ M in 100 μM cyclic GMP solution applied to the membrane, a level apparently too low for kinases to function. Phosphorylation supported by endogenous triphosphates in the membrane also seems unlikely in our experiments, because any triphosphates present during the first application of cyclic GMP would be washed out during

the repeated exchange of solution. We conclude that cyclic GMP acts directly on the channels in the membrane.

Because we could not detect current jumps corresponding to single-channel activity, the conductive properties of the ROS plasma membrane patches were determined from their integral voltage-current relations (Fig. 2). Ca^{2+} ions (10^{-8} – 10^{-3} M) had no effect on the conductance of the system in the absence of cyclic GMP. The cyclic GMP-dependent component of conductance decreased when the Ca^{2+} concentration was lowered; at 10^{-8} M Ca^{2+} it was 20–30% lower than that at 10^{-3} M Ca^{2+} .

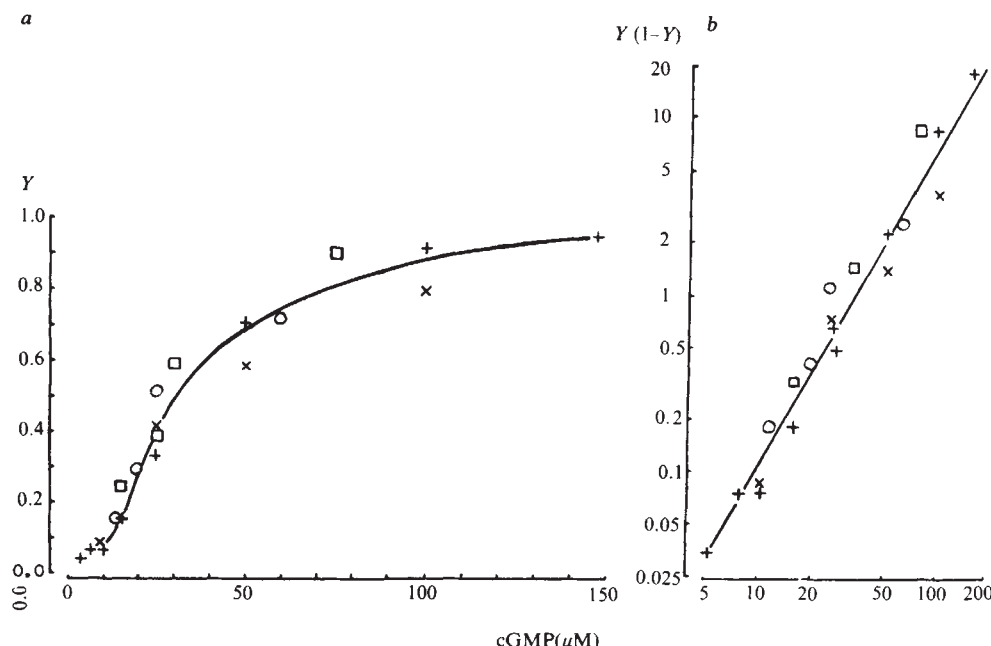
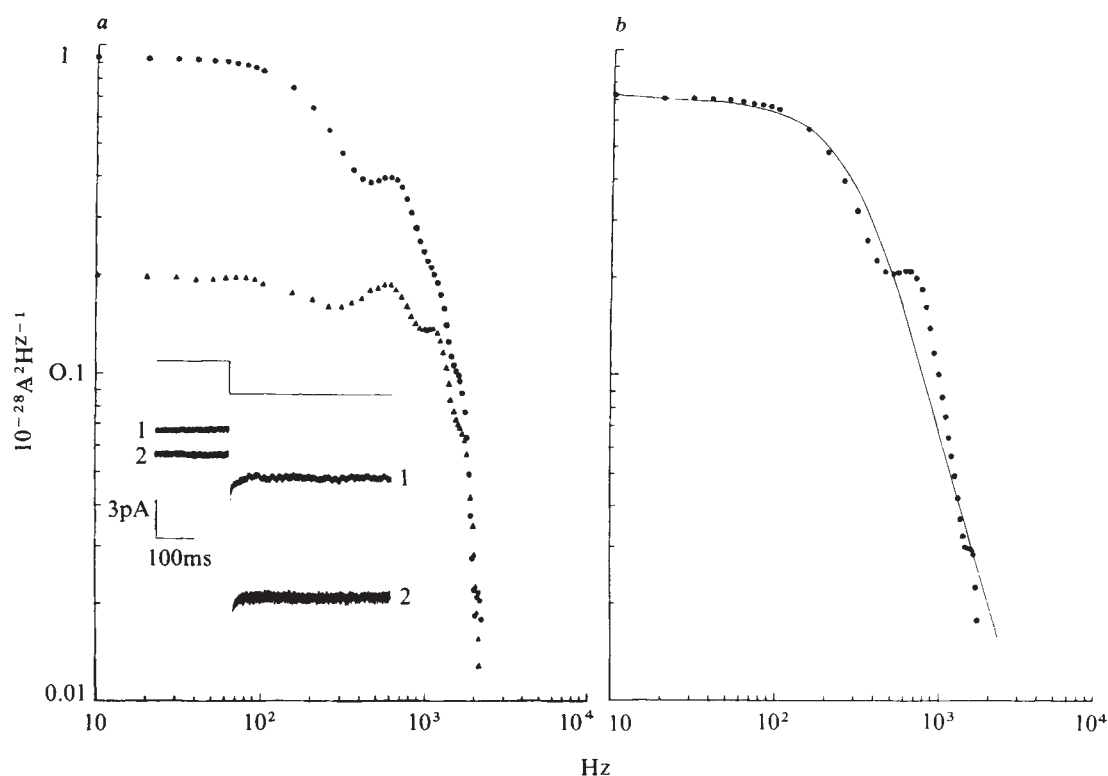


Fig. 3 *a*, Dependence of cyclic GMP-induced component of patch conductance, expressed in the normalized form (Y), on cyclic GMP concentration. The cyclic GMP-induced conductance at 300 μM cyclic GMP is taken as unity. As the voltage-current relations are nonlinear, the mean steady-current conductance in the range -20 to $+20$ mV was used. The data points represent single measurements on four different patches. $+$, 10 nM Ca^{2+} ; $\times$, $\circ$, $\square$, 0.1 mM Ca^{2+} . *b*, The same dependence in Hill's coordinates ($\log Y/(1-Y)$, $\log C$), where C is the cyclic GMP concentration.

Fig. 4 *a*, Power density spectra from a patch of the ROS plasma membrane at -30 mV. $\blacktriangle$, Background noise spectrum; $\bullet$, spectrum for current fluctuations in the presence of 100 μM cyclic GMP. Data were sampled at 50- μs intervals. Cyclic GMP was applied in standard Ringer. The insert shows the responses of the patch current to a 30-mV hyperpolarizing voltage jump. Curve 1, no cyclic GMP; curve 2, 100 μM cyclic GMP. *b*, Power density spectrum for the cyclic GMP-dependent current (after subtraction of background noise). The curve is a lorentzian function fitted by the least-squares method. The cut-off frequency of the lorentzian function is 316 Hz; the corresponding time constant is ~ 0.5 ms. The mean variance of the cyclic GMP-dependent current for the experiment illustrated was $\sim 3.5 \times 10^{-26} A^2$, and the change of the mean current on cyclic GMP application at -30 mV was 10.5 pA. The amplitude of an elementary event is $3.5 \times 10^{-26} A^2 / 10.5 \times 10^{-12} A = 3.3$ fA.



To test the ion selectivity of cyclic GMP-dependent conductance, we measured the reversal potentials for the cyclic GMP-dependent current when the intracellular surface of a patch was bathed with media of various ionic compositions, thereby enabling us to compare the data obtained from different patches showing different cyclic GMP-induced conductance. Our analysis reveals that cyclic GMP induces cationic conductance, because, when the NaCl gradient was doubled, the reversal potential obtained was equivalent to that of Na^+ ions (16.5 ± 0.3 mV, six determinations on different patches, Fig. 2b). Substitution of NaCl in the Ringer solution bathing the intracellular side of the membrane by KCl, LiCl, RbCl or CsCl resulted in

respective reversal potentials for the cyclic GMP-dependent current of 2.7 ± 0.7 mV, 2.2 ± 2.1 mV, 5 ± 1 and 6 ± 2.2 mV (8, 8, 4 and 4 determinations, respectively). These values indicate that the channels controlled by cyclic GMP are moderately selective for monovalent cations in the sequence $Na^+ > Li^+$, $K^+ > Rb^+$, $Cs^+ \gg Cl^-$.

The family of voltage-current relations for a single patch, obtained at different cyclic GMP concentrations (Fig. 2c), shows that the most marked conductance changes occur in the range of 20–60 μM cyclic GMP and that at 300 μM there is complete saturation of the cyclic GMP-dependent conductance. When studying the dependence of patch conductance on cyclic GMP

concentration, we therefore expressed the cyclic GMP-induced conductance in normalized form, taking the conductance at 300 μ M cyclic GMP as unity.

The curve showing the dependence of conductance on cyclic GMP concentration was S-shaped (Fig. 3a), with a Hill coefficient of ~ 1.8 (Fig. 2b) and a 50% effect at 30 μ M cyclic GMP. Neither Ca^{2+} ions nor 3',5'-cyclic AMP, 2',3'-cyclic GMP or 5' GMP (1 mM) had any effect on the cyclic GMP dependence of the conductance, confirming that cyclic GMP is a specific agent for inducing the membrane conductance increase.

The magnitude of the events underlying the cyclic GMP-induced conductance was estimated approximately by dividing the change in the current produced by cyclic GMP by the change in mean current⁷. The amplitude of the estimated unit event for three patches was ~ 3 fA ($\pm 10\%$) in standard Ringer solution at a membrane voltage of -30 mV. If it is assumed that the initial event is the closure of a single cyclic GMP-sensitive channel, then a single channel has a conductance of 100 fS. At this value, the channel responsible for the cyclic GMP-induced conductance could be either a carrier or a pore.

As the cyclic GMP-dependent current is not inactivated, at least within minutes, the power density spectrum of the steady-state current can be deduced. The spectra obtained at -30 mV in the absence and presence of cyclic GMP are shown in Fig. 4a. The spectrum for the cyclic GMP-dependent current with the best least-square lorentzian function fit (Fig. 4b) indicated a cut-off frequency of ~ 300 Hz, corresponding to a time constant of ~ 0.5 ms.

It is known that the extracellular and intracellular application of cyclic GMP depolarizes the rod in the dark^{1,2,8}, probably by increasing the light-sensitive component of the dark conductance of the plasma membrane⁹. This suggests that the light-controlled conductivity units are activated by cyclic GMP. (We do not know whether the properties of the cyclic GMP-activated conductance we have studied *in vitro* are the same as those of the light-sensitive conductance recorded *in vivo*.)

It has been shown recently that the photocurrent through the rod plasma membrane previously thought to be dependent on Na^+ ions¹⁰ can also be carried by Li^+ , K^+ , Rb^+ , Cs^+ , Ti^+ , Ca^{2+} , Ba^{2+} and Sr^{2+} ions¹¹. The selectivity for monovalent cations found by us corresponds to that reported elsewhere¹¹. The disappearance of photoresponses in the absence of Na^+ observed previously results from the rise in the intracellular Ca^{2+} concentration due to inactivation of the Na^+ - Ca^{2+} exchanger¹¹⁻¹⁵.

Estimates of the single-event light-sensitive conductance for the rod plasma membrane of the turtle, lizard and salamander are 600, 50 and 5 fS, respectively¹⁶⁻¹⁸, the same order of magnitude as the initial event of the cyclic GMP-dependent conductance we describe. Information on the power density spectrum of the light-dependent current in rods is very limited and only two values for the time constant are available, 200 ms for the turtle rod¹⁶ and 0.8 ms for the salamander¹⁸. The reason for this discrepancy between the two estimates is unclear. We consider it unlikely that the ensemble of channels with intrinsic time constants of the order of hundreds of milliseconds could produce responses to bright flashes which would develop within milliseconds¹⁹. Therefore, the lower value, which is consistent with our estimate of 0.5 ms, seems the more reliable.

In view of the similarities between the properties of the light-dependent conductance of the ROS membrane and those of the cyclic GMP-dependent conductance of the rod membrane patches, it is quite feasible that the cyclic GMP-activated conductances we describe are responsible for the light-dependent conductance of the ROS membrane. If this is so, cyclic GMP, not Ca^{2+} , is the messenger modulating the conductance of the plasma membrane. The effect of Ca^{2+} on the rod photoresponse may be through its action on the cyclic GMP metabolism in the photoreceptor. A decrease in Ca^{2+} concentration is known to produce a marked increase in intracellular cyclic GMP concentration in the rod^{20,21}, which could be explained by a direct effect of Ca^{2+} on ROS cyclic GMP phosphodiesterase²².

The direct action of a cyclic nucleotide on the conductance of the plasma membrane has not previously been described. The advantage of such a mechanism is its fast response time, essential in photoreceptors, where the response to intense illumination develops within milliseconds and single-channel open times are less than 1 ms. It will be exciting to see whether the same mechanism exists in other cells.

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Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C

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One of the molecular mechanisms capable of regulating the physiological properties of neurones is the phosphorylation of ion channels and other cellular components by cyclic AMP-dependent protein kinase¹. Another protein kinase present in high concentrations in the mammalian brain is protein kinase C (a calcium/phosphatidylserine/diacylglycerol-dependent protein kinase)²⁻⁵, but there is no direct evidence, as yet, for the involvement of this enzyme in the control of neuronal excitability. We now present evidence that activation of endogenous protein kinase C by the tumour-promoting phorbol ester TPA (12-O-tetradecanoyl-phorbol-13-acetate), or intracellular injection of the purified enzyme, enhances the voltage-sensitive calcium current in bag cell neurones of the mollusc *Aplysia*.

Previous experiments have demonstrated that protein kinase C is present in broken cell preparations of the bag cell neurones and that 10-100 nM TPA, but not non-tumour-promoting phorbols such as 4- α -phorbol^{6,7}, could activate the enzyme. In the present study, exposure of bag cell neurones to doses of TPA between 10 and 100 nM consistently enhanced the height of evoked action potentials (Fig. 1, Table 1); this effect took several minutes to develop and its magnitude was independent of the

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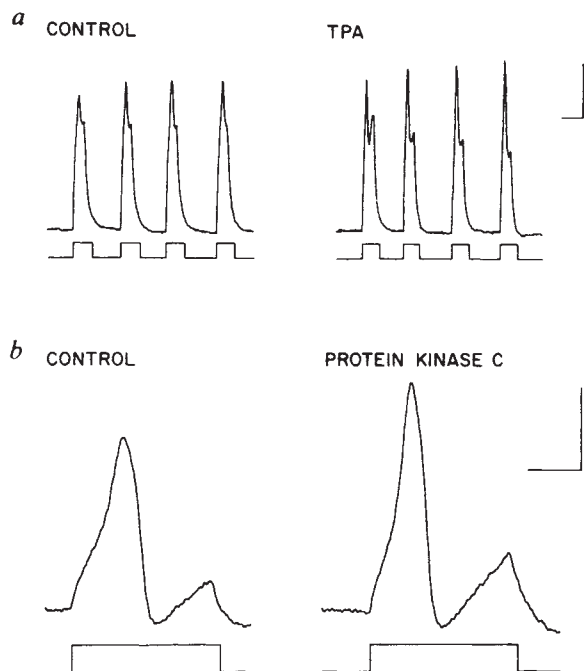


Fig. 1 TPA (a) and protein kinase C (b) each enhance the height of bag cell neurone action potentials. Bag cells were dissociated and maintained in primary cell culture for 1–14 days as described previously¹⁴. Intracellular recordings were made with a single electrode connected to a bridge circuit for current injection. Action potentials were elicited by applying pulses of depolarizing current and data were recorded with a Nicolet digital oscilloscope and a chart recorder. Measurements of action potential characteristics before and after treatment were made at the same membrane potential; where necessary, d.c. was injected to adjust the membrane potential before stimulation. *a*, Effect of TPA. The recording electrode was filled with 3 M KCl. TPA (Sigma) was dissolved in dimethyl sulphoxide (DMSO). Final concentrations of TPA and DMSO in the bath were 10 nM and 0.0005% respectively. (This concentration of DMSO had no effect in control experiments.) The membrane potential of the cell shown was -66 mV. The stimulus was a 200-ms pulse of 0.65 nA. Scale bars: 20 mV, 300 ms. *b*, Effect of injection of protein kinase C. Protein kinase C was purified from bovine brain by a modification of the method of Kikkawa *et al.*¹⁵. The electrode tip was filled with protein kinase C ($16 \mu\text{g ml}^{-1}$) in 20 mM potassium phosphate buffer (pH 7.2), 0.9 M KCl. The enzyme was pressure-injected with a 2-min pulse of 50 p.s.i. The membrane potential of the cell shown was -30 mV. The stimulus was a 500-ms pulse of 0.11 nA. Scale bars: 20 mV, 200 ms.

concentration of TPA over the range tested. No significant changes in the width of the action potential, the amplitude of the afterpotential following an action potential, or the apparent input resistance were found. The inactive 4- α -phorbol had no effect on any parameters of the evoked action potential.

Injection of purified protein kinase C mimicked the effect of TPA on the height of the action potential (Fig. 1*b*). Control injections of either heat-inactivated enzyme or carrier solution alone had no effect (Table 1). These results are consistent with the hypothesis that the effects of TPA on the electrical properties of the bag cells are mediated by the activation of protein kinase C.

The size and shape of action potentials are determined by the properties of several distinct ionic conductances. In order to determine which currents were affected, the effects of TPA on both inward and outward currents were examined in voltage-clamped, internally dialysed bag cell neurones. Figure 2 shows that the peak inward current in voltage-clamped bag cell neurones internally dialysed with K^+ -current blockers was twice as large, on average, in cells pretreated with TPA as that in control cells from the same ganglion. TPA had no apparent effect on either the voltage dependence of activation or the

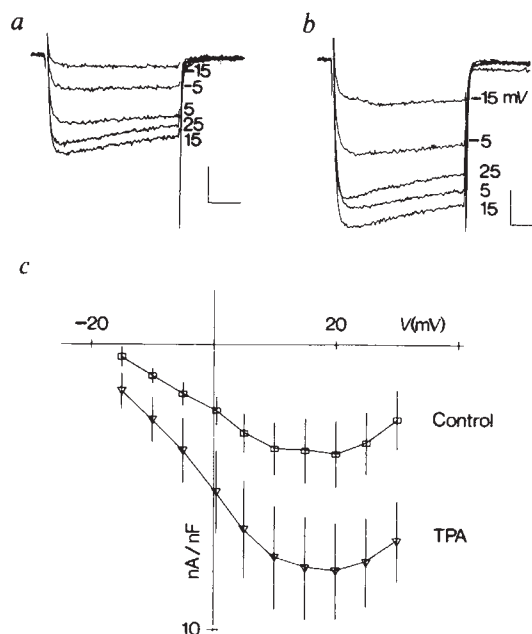


Fig. 2 Pretreatment with TPA enhances net inward current in voltage-clamped, internally dialysed bag cell neurones. *a*, Inward currents seen in a control cell which had been treated with 4- α -phorbol for 40 min before the beginning of intracellular dialysis. Cell capacitance, 0.095 nF. Scale bars: 0.25 nA, 20 ms. *b*, Inward currents measured in another cell (from the same ganglion as the cell in *a*) which had been treated with TPA for 55 min before the beginning of intracellular dialysis. Cell capacitance, 0.07 nF. Scale bars as in *a*. *c*, Mean *I-V* (current-voltage) curve from a set of experiments such as those shown in *a* and *b*. Peak inward currents were normalized by cell capacitance (estimated by integrating the unfiltered current transient seen during a brief hyperpolarizing pulse). Mean values of peak inward current density are presented ($\pm$ s.e.m.) for seven cells pretreated with 4- α -phorbol for 10–60 min ($\square$, control) and for seven paired cells pretreated with TPA for 30–75 min (∇). The peak inward current was significantly greater in the TPA-pretreated cells ($P < 0.01$, $t = 3.267$). There were no significant differences in capacitance or resistance between the two groups of cells. In three of seven TPA-treated cells and three of seven control cells, the inward current also contained a small, rapidly inactivating component which was probably a tetrodotoxin-sensitive sodium current (not shown).

Methods. Bag cells were dissociated and maintained for 1–2 days in primary culture. The cells were plated into plastic Petri dishes (Falcon) which had been treated to prevent the cells from adhering to the dish and growing neurites. These 'floating' cells had ionic currents similar to those seen in attached, growing cultured cells, but faster dialysis and more stable recordings were possible. Cells were exposed to either the inactive phorbol, 4- α -phorbol (control), or the active phorbol ester, TPA, before and during patch-clamping and dialysis. Final concentrations of 4- α -phorbol, TPA and DMSO in the bath were 100 nM, 100 nM and 0.005%, respectively. Each TPA-treated cell was matched with a control cell from the same ganglion, examined after the same number of days in primary culture. The whole-cell patch-clamp method was used to voltage-clamp and internally dialyse the cells¹⁶. The internal dialysis solution used for recording inward currents contained (in mM): 100 tetraethylammonium (TEA)-aspartate, 453 Cs-aspartate, 17 Cs-phosphate, 10 reduced glutathione, 5 Na₂ATP, 0.1 Na₂GTP, 5 MgCl₂, 20 HEPES (pH 7.3), 20 EGTA, 4.14 CaCl₂ and 1 mg ml⁻¹ glucose. With this internal solution, outward currents were reduced during the first 1–2 min of dialysis, leaving a net inward current which could be observed for over 30 min, but the amplitude of which gradually declined (20–70% decrease in 30 min), as reported by others¹⁷. Therefore, in these experiments inward currents were recorded at equivalent times, starting 3–4 min after the onset of dialysis. Currents were elicited by depolarizing pulses from the holding potential (-60 mV) to the indicated potential, followed by repolarization to -40 mV. Inter-stimulus interval was 30 s. Current records were filtered (4,000 Hz) and digitized (256 points per record); points are connected with straight lines. The ohmic portion of the leakage current (calculated from resistance measured during small hyperpolarizing voltage steps) has been subtracted.

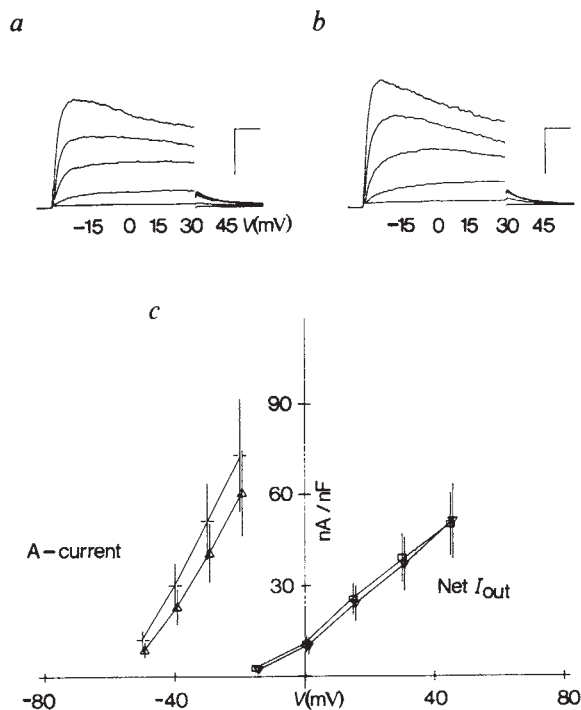


Fig. 3 Pretreatment with TPA does not change net outward current (I_{out}) in voltage-clamped, internally dialysed bag cell neurones. Cells were cultured, voltage-clamped and internally dialysed as described in Fig. 2 legend, except that the internal dialysis solution contained K^+ instead of Cs^+ and TEA^+ . Calcium-activated potassium currents were blocked by the 20 mM EGTA in the internal solution. *a*, Outward currents seen in a control cell during 175-ms depolarizing pulses from -60 mV to the indicated voltages, followed by repolarization to -40 mV. Inter-stimulus interval 60 s. Cell capacitance, 0.10 nF. Currents are shown as in Fig. 2, except that no leak subtraction was used. Scale bars: 3 nA, 30 ms. *b*, Outward currents seen in another cell (from the same ganglion as the cell in *a*) which had been pretreated with 100 nM TPA for 16 min; the same protocol was used as in *a*. Cell capacitance, 0.10 nF. Scale bars as in *a*. *c* (Right), mean ($\pm$ s.e.m.) net outward current density at the end of 175-ms depolarizing pulses (as in *a* and *b*) in six control cells ($\square$) and six paired TPA-pretreated cells (∇). Control cells were pretreated with either 100 nM 4- α -phorbol (final concentration in DMSO = 0.005%, $n = 2$) or 0.05% DMSO alone ($n = 4$). Left, mean ($\pm$ s.e.m.) peak A-current (transient potassium current) observed in the same cells used for measurement of net outward current ($+$, control; Δ , TPA-pretreated); no sample records are shown. A-current was measured during depolarizations to the indicated voltages; inactivation was removed with 1-s prepulses to -100 mV from the holding potential of -60 mV. There were no significant differences in either net outward current or A-current at any potential. Three of six TPA-treated cells had a marked net inward current at the beginning of the depolarization (examples not shown).

kinetic properties of the inward current, although these were not examined in detail.

The predominant inward current in cultured bag cell neurones is carried by calcium ions⁸. Several lines of evidence suggest that the TPA-induced current observed here is also carried by calcium. In one experiment, addition of the calcium channel blocker lanthanum (1 mM) to the bath completely abolished the inward current in a TPA-pretreated cell. Furthermore, in experiments in which *N*-methylglucamine was substituted for sodium in the extracellular solution, the inward current was still enhanced by pretreatment with TPA ($n = 5$ TPA-treated cells, 5 matched control cells; $P < 0.05$). These results, and the similarity in the kinetics and voltage dependence of the TPA-induced current to the predominantly calcium-carried control current (Fig. 2), indicate that TPA enhances a calcium current in the bag cell neurones.

Table 1 Effects of TPA and protein kinase C on action potentials of bag cell neurones

	<i>n</i>	% Change in height of action potential	% Change in width of action potential
TPA	10	$+21.3 \pm 3.5^*$	-8.3 ± 18.0
Protein kinase C	15	$+10.4 \pm 4.0^\dagger$	$+3.2 \pm 8.8$
Control injections	13	$+1.5 \pm 2.5$	12.0 ± 7.1

TPA was added to final concentrations of 10 nM ($n = 4$), 50 nM ($n = 3$) or 100 nM ($n = 3$). Protein kinase C was injected in a carrier solution of 20 mM potassium phosphate buffer (pH 7.2), 0.9 M KCl ($n = 15$). Control injections comprised carrier solution alone ($n = 8$) or protein kinase C (in carrier solution) which had been inactivated by heating at 50 °C for 90 min ($n = 5$). No differences between the two control groups were noted. Action potential widths were measured at two-thirds peak height. In addition to the parameters of the action potentials, the afterhyperpolarization following an action potential and the input resistance of the cells were monitored; no significant changes in the latter two parameters were observed for any of these treatments. Significance was calculated using a paired *t*-test.

* $P < 0.03$; $^\dagger P < 0.02$.

The effects of pretreatment with TPA on voltage-dependent potassium currents in the bag cell neurones were also examined (Fig. 3). TPA pretreatment caused no obvious changes in the amplitude, kinetics or activation parameters of the net outward currents observed at voltages between -15 and $+45$ mV or of the transient outward current (A-current). TPA has been proposed to enhance calcium-dependent potassium currents in lymphocytes⁹; we have not yet determined whether TPA has an effect on calcium-dependent potassium currents in bag cell neurones.

Although TPA consistently enhanced inward currents when cells were exposed to the drug before internal dialysis, we observed no effects when TPA was added to the bath after dialysis had begun ($n = 6$ cells with the potassium-blocking solutions and $n = 4$ cells with potassium aspartate solutions, including solutions in which the EGTA and calcium concentrations were altered). In broken cell preparations of bag cell neurones, TPA can stimulate protein phosphorylation in the presence of these internal dialysis solutions. It seems, therefore, that internal dialysis disrupts some intracellular process required for the enhancement of the calcium current by TPA. Once established, however, the enhancement effect is not abolished by internal dialysis.

The results presented here suggest that the TPA-induced activation of protein kinase C in bag cell neurones can lead to an increase in the voltage-dependent calcium current without altering the potassium currents examined. In contrast, activation of cyclic AMP-dependent protein kinase causes decreases in at least three potassium currents in the same cells without affecting the inward currents^{8,10,11}. Therefore, both protein kinase C and cyclic AMP-dependent protein kinase enhance action potentials, but they do so by affecting the activity of different ion channels. The enhancement of calcium entry by a calcium-sensitive enzyme, protein kinase C, may produce an excitatory, positive feedback mechanism in the bag cell neurones. A similar positive feedback mechanism has been proposed for ventricular muscle¹³. Such feedback could contribute to the prolonged electrical after-discharge and associated enhancement of action potential height which can be induced by brief stimulation of intact clusters of bag cell neurones¹².

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Charybdotoxin, a protein inhibitor of single Ca^{2+} -activated K^+ channels from mammalian skeletal muscle

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The recent development of techniques for recording currents through single ionic channels¹ has led to the identification of a K^+ -specific channel that is activated by cytoplasmic Ca^{2+} (refs 2-12). The channel has complex properties, being activated by depolarizing voltages and having a voltage-sensitivity that is modulated by cytoplasmic Ca^{2+} levels. The conduction behaviour of the channel is also unusual, its high ionic selectivity being displayed simultaneously with a very high unitary conductance^{2,4,12}. Very little is known about the biochemistry of this channel, largely due to the lack of a suitable ligand for use as a biochemical probe for the channel. We describe here a protein inhibitor of single Ca^{2+} -activated K^+ channels of mammalian skeletal muscle. This inhibitor, a minor component of the venom of the Israeli scorpion, *Leiurus quinquestriatus*, reversibly blocks the large Ca^{2+} -activated K^+ channel in a simple bimolecular reaction. We have partially purified the active component, a basic protein of relative molecular mass (M_r) ~7,000.

In all the experiments described here, we measured K^+ currents through single Ca^{2+} -activated K^+ channels from rat skeletal muscle plasma membranes inserted into planar phospholipid bilayers. Latorre and colleagues^{4,9,11} have shown that the native functions of this channel are preserved on transfer into a planar bilayer in minimal conditions using aqueous solutions containing only inorganic ions and buffers.

Figure 1a illustrates the effect of crude *L. quinquestriatus* venom (LQV) on the Ca^{2+} -activated K^+ channel in planar bilayers. In control conditions the channel was 'active' for almost all the time (that is, it opened and closed rapidly on a 10-ms time scale) and was closed for ~80% of the time (as shown in the expanded-scale trace). Single-channel activity of this kind will continue for hours if precautions are taken to prevent incorporation of more than one channel into the bilayer. Addition of LQV to the external solution caused an obvious change in the behaviour of the single channel, when examined on a slow time scale: long-lived nonconducting states separating 'bursts' of active channels were apparent. Within such a burst, the rapid opening and closing of the channel and its unitary current were identical to those seen in control conditions. The venom exerts its inhibitory effect only when added to the 'extracellular' solution, that is, the side opposite to that on which Ca^{2+} activates the channel. We next addressed two questions:

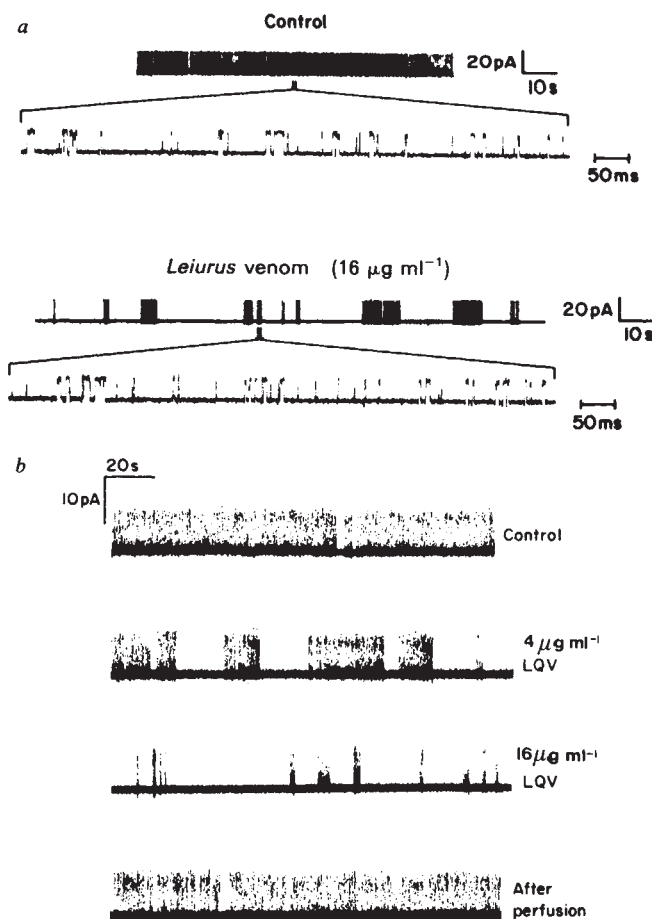


Fig. 1 Effect of *Leiurus* venom on the Ca^{2+} -activated K^+ channel. **a**, Control record taken for ~2 min, after which LQV (16 $\mu\text{g ml}^{-1}$; Sigma) was added to the external solution, and records were collected for a further 2 min. All traces were recorded with continual stirring. A 200-fold expanded-time trace is displayed below each record to illustrate the channel's gating. **b**, Concentration-dependence and reversibility of *Leiurus* venom. The traces show channel behaviour before addition of crude venom (top trace), in the presence of venom at the indicated concentrations (middle two traces), and after extensive perfusion of the external chamber with venom-free medium.

Methods: A planar bilayer was formed from a decane solution of phosphatidylethanolamine (24 mM)/phosphatidylcholine (6 mM), and a single Ca^{2+} -activated K^+ channel from rat muscle transverse (T)-tubule vesicles, prepared as described elsewhere²³, was inserted into the bilayer by the 'fusion' method⁴. The 'external' aqueous solution of the bilayer system, topologically equivalent to the external face of the channel in its native membrane, contained (in mM): 5 KCl, 90 NaCl, 0.2 EGTA, 10 MOPS-KOH, pH 7.0. The 'internal' solution contained (in mM): 195 KCl, 0.08 CaCl_2 , 10 MOPS-KOH, pH 7.0. Incorporation of the channel was effected by adding rat T-tubule vesicles (2 $\mu\text{g ml}^{-1}$) to the internal side of the bilayer, but without the NaCl on the external side. After the appearance of the channel, NaCl was added to the external side, to the final concentration given above. Single-channel records were collected on FM tape at a holding voltage of 20 mV (cellular convention, with external solution ground). Channel openings, representing outward K^+ current, are upward.

- (1) What is the nature of the active toxin in the venom? (2) What is the mechanism of inhibition of the Ca^{2+} -activated K^+ channel by the toxin?

Figure 1b shows the concentration-dependence of the effect of LQV: with a rise in concentration of LQV, the inhibition of the channel became increasingly pronounced, as seen by the shortening of the active bursts. The average dwell times in the LQV-induced nonconducting states did not change with venom concentration (see below). The effect of the venom was readily

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reversed by perfusing the external side of the bilayer with venom-free medium. These characteristics are reminiscent of a slow-blocking mechanism of channel inhibition, in which the active toxin in the venom, TX, associates reversibly with the active channel in a bimolecular fashion^{11,13}:



This scheme interprets each long-lived blocked state observed in the single-channel record as resulting from an interval of time in which a single toxin molecule is associated with a single channel. The scheme makes several strong predictions: both active and blocked dwell times must be exponentially distributed and the average blocked time, τ_b , must be independent of toxin concentration, as

$$1/\tau_b = k_{\text{off}} \quad (2)$$

Moreover, the average time in the active state, τ_a , must be inversely proportional to toxin concentration, as

$$1/\tau_a = k_{\text{on}}[\text{TX}] \quad (3)$$

As shown below, these predictions are satisfied.

The validity of equation (3) is especially important, as it allows us to develop a linear assay for the active component of the crude venom. We used the toxin-induced shortening of the active dwell time of single Ca^{2+} -activated K^+ channels as a functional assay to fractionate the venom and purify the channel inhibitor.

A two-step fractionation of LQV yielded partially purified toxin (Fig. 2). Cation-exchange chromatography of crude venom on Bio-Rex 70 produced multiple protein peaks, but channel-inhibiting activity was observed only at high salt elution ($>500 \text{ mM}$; Fig. 2a). When such high-salt fractions were applied to a Bio-Gel P10 column, two peaks of protein were observed, but only the more rapidly moving peak had toxin activity; selected assays of these 'P10' fractions are shown in Fig. 2b, demonstrating that the single-channel assay of toxin activity can be wholly quantitative. This Bio-Rex 70/Bio-Gel 10 fractionation results in a recovery of $\sim 70\%$ of total toxin activity with 1–2% of the protein, a 50-fold purification. Our best estimates indicate that the P10 fraction is $\sim 30\%$ pure, comprising a prominent band of $M_r \sim 7,000$ (7K) on SDS-polyacrylamide gradient gels, with higher molecular weight contaminants.

This partially purified toxin is directed against a channel which, because of its high conductance and selectivity, has been described structurally as analogous to a funnel with a huge mouth¹². Similarly, we may view the channel's structure as that of a giant whirlpool, and accordingly name the agent in LQV directed against it 'charybdotoxin' (CTX)¹⁴. The CTX activity is sensitive to washing, to reduction with dithiothreitol, and to treatment with chymotrypsin (data not shown). The activity is dialysable through 10K cutoff tubing but is retained in 3.5K cutoff tubing. We therefore envisage CTX as a small, basic, disulphide-linked protein, like other scorpion neurotoxins¹⁵. The purified toxin II α from *L. quinquestriatus* (given by Dr Gary Strichartz), a potent modifier of Na^+ channels^{16,17}, has no effect on the Ca^{2+} -activated K^+ channel studied here.

We next examined the mechanism of action of the partially purified CTX from the Bio-gel P10 column. As both active and blocked states are distributed exponentially (data not shown), the mean dwell times in the active and blocked states are a measure of the rate constants of blocking and unblocking, respectively. When the CTX concentration was varied, the mean dwell times behaved as expected for a simple bimolecular process (Fig. 3). The inverse of the mean time in the active state varied linearly with CTX concentration, whereas the blocked time was independent of CTX concentration [equations (2) and (3)]. Thus, CTX blocks the Ca^{2+} -activated K^+ channel in a manner similar to that by which tetrodotoxin and saxitoxin block Na^+ channels of excitable membranes^{13,18}.

These experiments do not elucidate the mechanism by which

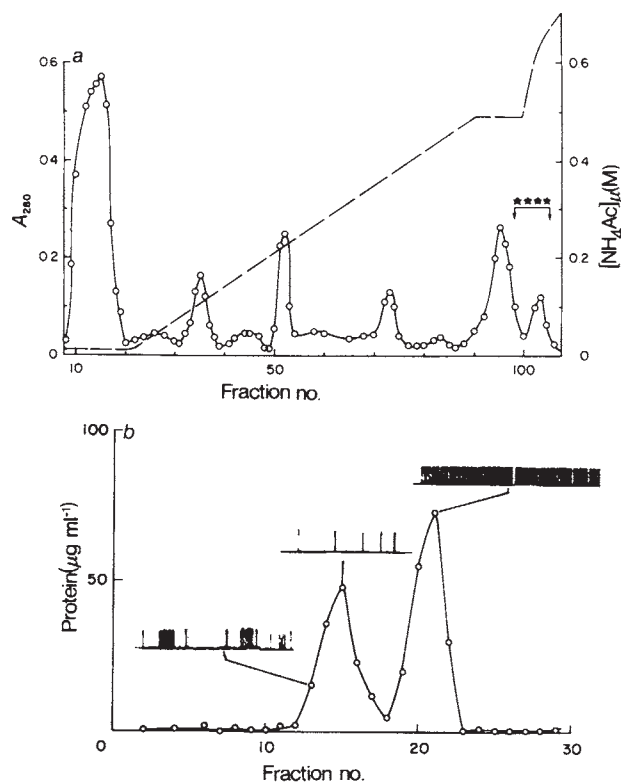


Fig. 2 Fractionation of *Leiurus* venom. Crude *Leiurus* venom was dissolved at 1 mg ml^{-1} in 10 mM $\text{NH}_4\text{-acetate}$, $\text{pH } 7$ and centrifuged at $30,000 \text{ g}$ for 15 min . **a**, The supernatant (7 ml) was applied to a 10-ml Bio-Rex 70 column (400 mesh), equilibrated in 10 mM $\text{NH}_4\text{-acetate}$, and eluted with the salt gradient shown at a flow rate of 0.2 ml min^{-1} . Fraction size 0.9 ml . Fractions were assayed for activity by the ability of a $30\text{-}\mu\text{l}$ sample to inhibit Ca^{2+} -activated K^+ channels in planar bilayers. All regions of the elution profile were assayed, and only the fractions marked by asterisks had measurable activity. **b**, The high-salt fraction obtained from the Bio-Rex 70 column in **a** was lyophilized and dissolved in 0.5 ml , then applied to a Bio-Gel P10 column (10 ml), equilibrated in 100 mM NaCl , 5 mM NaPi , $\text{pH } 7.0$. The column was run at 0.1 ml min^{-1} , and 0.3 ml fractions were collected. Protein was determined by the fluorescamine assay²⁴, with bovine serum albumin as the standard. The figure shows representative single-channel traces to illustrate the assay of toxin activity; for each record, a Ca^{2+} -activated K^+ channel was placed in a planar bilayer, and recordings were taken after addition of $20 \text{ }\mu\text{l}$ of the indicated fraction. Channel assay conditions were as in Fig. 1, except that the NaCl was omitted from the external solution; this increases the sensitivity of the assay ~ 10 -fold.

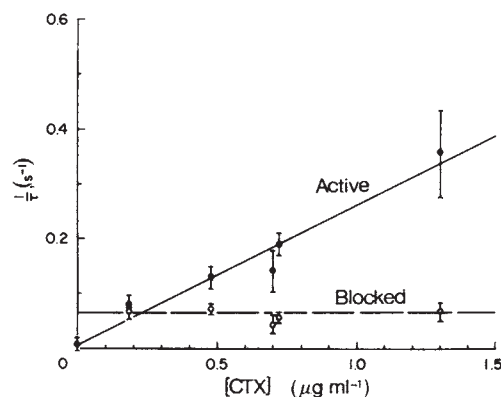


Fig. 3 The blocking kinetics of charybdotoxin, prepared as in the two-step fractionation of Fig. 2, were examined in conditions described for Fig. 1. At each CTX concentration, records were taken for $30\text{--}150 \text{ min}$, and mean dwell times were calculated. Each point represents the mean time ($\pm \text{s.e.m.}$) calculated from $30\text{--}300$ transitions on a single-channel membrane.

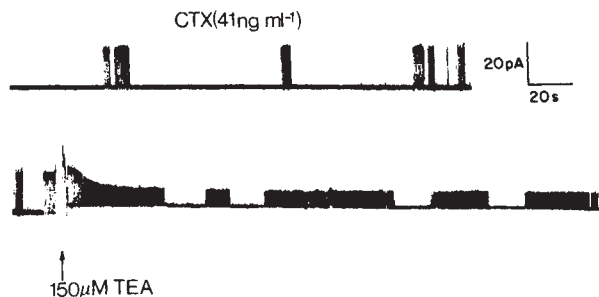


Fig. 4 Release of CTX blockade by tetraethylammonium (TEA). The activity of a single Ca^{2+} -activated K^+ channel was recorded in the conditions described for Fig. 1, except that NaCl was omitted from the external bath. After obtaining a control record (not shown), 41 ng ml^{-1} CTX was added, and ~ 30 blocking events were recorded, a representative sample of which is shown (top trace). TEA ($150 \mu\text{M}$) was added at the arrow, and data were collected for a further 5 min. The diffusion of TEA up to the membrane surface can be seen as a reduction of the channel height. Mean dwell times in the active state were $3.9 \pm 0.6 \text{ s}$ and $12 \pm 2 \text{ s}$, before and after addition of TEA, respectively.

CTX binding to the Ca^{2+} -activated K^+ channel leads to a long-lived nonconducting state. There are two obvious candidates: direct occlusion of the channel's conduction pathway, and interaction with the channel's gating machinery. The experiment shown in Fig. 4 supports strongly the direct occlusion mechanism. We exploited the fact that this channel is blocked from the external side by tetraethylammonium ion (TEA) at submillimolar concentrations^{19,20}. Figure 4 shows that addition of external TEA not only caused a reduction in K^+ current through the channel (as expected for a rapidly reversible blocking reaction), but also relieved inhibition of the channel by CTX. Thus, there is a strong interaction between TEA and CTX on the external side of the channel. As TEA is known to block many K^+ channels by directly entering the conduction pathway¹², the CTX block is probably due to channel occlusion by binding at a site near the TEA receptor in the external mouth of the channel.

This represents the first report of a high-affinity inhibitor of the high-conductance Ca^{2+} -activated K^+ channel. We can estimate the potency of CTX from Fig. 3, assuming a molecular weight of 7,000 and a purity of 30%. In these conditions the K_D for association with the Ca^{2+} -activated K^+ channel is $\sim 10 \text{ nM}$. This high affinity, combined with the long blocked time ($\sim 10 \text{ s}$ at room temperature), makes CTX a suitable candidate for probing and possibly purifying the Ca^{2+} -activated K^+ channel. It could be used as binding ligand, a photoaffinity ligand, or possibly as a basis for affinity chromatography.

At present, we have little information about the specificity of CTX. We know that it has no effect on K^+ channels from mammalian sarcoplasmic reticulum (unpublished data), on batrachotoxin-activated Na^+ channels from rat muscle (unpublished data), or on K^+ channels of squid axon (W. Gilly, unpublished results). These initial results suggest that CTX may indeed be specifically targeted against the Ca^{2+} -activated K^+ channel, and we are continuing to investigate this specificity.

To avoid possible confusion, we should mention that apamin, a protein toxin of honeybee venom, was shown previously to inhibit Ca^{2+} -activated K^+ currents in neuroblastoma²¹. This toxin has no effect on the Ca^{2+} -activated K^+ channel studied here^{20,22}. This is not a contradiction, however, as it has been argued²² that apamin is specific for a different Ca^{2+} -activated K^+ channel, one with a low conductance ($\sim 20 \text{ pS}$) whose selectivity has not been studied in detail.

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Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester

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Although it has been proposed that the activation of T lymphocytes is mediated by an early rise in cytosolic calcium concentration¹⁻⁴, it has not been possible to mimic antigen- or mitogen-induced mouse lymphocyte activation by calcium ionophores that bypass receptor-mediated processes^{1,3}. There is now evidence from other systems that the rise in cytosolic calcium which follows receptor triggering is preceded by the breakdown of phosphatidylinositol biphosphate into 1,2-diacylglycerol and inositol trisphosphate (for reviews see refs 5-8). The latter is known to cause release of calcium from intracellular stores⁸. The cellular target for the former is now widely accepted to be protein kinase C (ref. 6). Therefore, ligand-induced cellular response follows a rise in cytosolic calcium concentration and protein kinase C activation. Here we confirm that the calcium ionophores A23187 and ionomycin do not activate mouse T lymphocytes. However, either one in combination with the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), which is structurally related to 1,2-diacylglycerol, induces in lymphoid cell populations the expression of receptors for interleukin-2 (IL-2), the secretion of IL-2 and cell proliferation as measured by ³H-thymidine uptake. The growth-promoting effect of IL-2^{9,10} on an exogenous IL-2-dependent clone could not be substituted for by ionomycin either alone or with TPA. Thus, the combination of calcium ionophores and TPA bypasses the requirement for antigen- or lectin-induced signal at the onset of lymphocyte activation.

T lymphocytes¹¹ from CBA mice were incubated with various concentrations of ionomycin, A23187 or concanavalin A (Con A) and in the presence or absence of TPA. After 3, 20 or 40 h of incubation, the supernatant fluid was removed and replaced with fresh culture medium. All wells were pulsed with ³H-thymidine after 40 h and cells collected 8 h later. Figure 1 shows that in the presence of 10 nM TPA, both ionomycin and A23187 induce a dose-dependent incorporation of ³H-thymidine, but that none of the compounds has any effect alone. TPA also enhances the Con A-induced response. Blast transformation is

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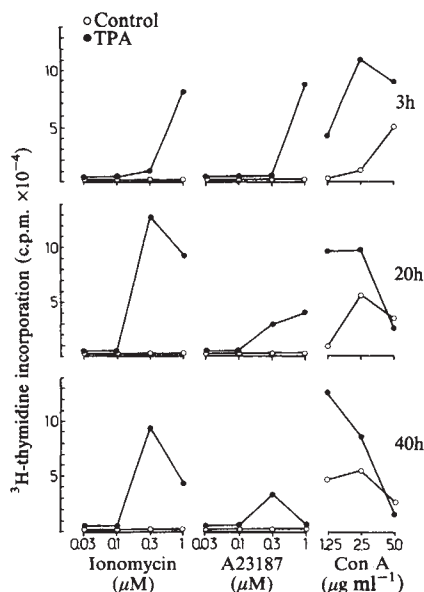


Fig. 1 Incorporation of ^3H -thymidine by mouse T lymphocytes after stimulation by calcium ionophores or Con A with or without TPA. T lymphocytes from spleen of CBA mice were obtained by filtration on nylon wool¹¹ and suspended in culture medium (RPMI 1640 medium supplemented with 5% fetal calf serum (Flow Laboratories), 2 mM glutamine, 0.5 μM 2-mercaptoethanol, penicillin and streptomycin); aliquots were placed in 96-well microtitre plates at 10^5 cells per well. Con A or the calcium ionophores A23187 or ionomycin (diluted from 5 mM stock solution in dimethyl sulphoxide) were added to give the desired final concentration (ionomycin kindly provided by Dr A. Mattei of Squibb Laboratories). TPA (10 nM) was added where appropriate. After 3, 20 or 40 h of incubation under a humidified and conditioned atmosphere (37°C, 7% CO_2), the plates were centrifuged, then the supernatant removed and replaced with fresh culture medium. After a total of 40 h of incubation, all wells were pulsed with 0.5 μCi of ^3H -thymidine (Amersham) and cells collected 8 h later. ^3H -thymidine incorporation was measured with a β -counter. Cells maintained in culture medium or treated with TPA alone always incorporated $<5,000$ c.p.m. Values are means of triplicates and are representative of several experiments.

also observed in all cultures where there is increased ^3H -thymidine uptake (data not shown). The maximally effective concentration of ionomycin and A23187 decreases with increasing incubation time before the medium is replaced. A long period of incubation (40 h) causes a decrease or abolition of ^3H -thymidine incorporation at the highest concentrations of ionomycin and A23187, as is also the case for Con A¹². All subsequent experiments were conducted using ionomycin as a stimulant in preference to A23187, because of the known higher specificity for calcium of ionomycin^{13,14} and because we find ionomycin to be more effective than A23187 at lower concentrations.

To show that activation by the calcium ionophores in conjunction with TPA is indeed calcium-dependent, T lymphocytes were incubated in medium containing the calcium chelator EGTA and challenged with ionomycin. After 3 or 20 h, the supernatant fluid was removed and replaced by fresh culture medium. Figure 2 shows that the presence of EGTA completely abolishes the ability of ionomycin to trigger ^3H -thymidine incorporation. This effect can be reversed by including excess CaCl_2 , but not MgCl_2 , during incubation with EGTA.

Mouse spleen T lymphocytes stimulated by ionomycin and TPA secrete IL-2 in the first 24 h after activation (Fig. 3). Supernatant was removed from cells 24, 48 or 72 h after treatment with the various mitogenic agents or medium alone and tested for activity on a T-lymphocyte clone, CTL-L¹⁵, which requires exogenous IL-2 for growth. Figure 3 shows that ionomycin in combination with TPA induced IL-2 secretion whereas neither ionomycin nor TPA had any effect alone. After 48 h,

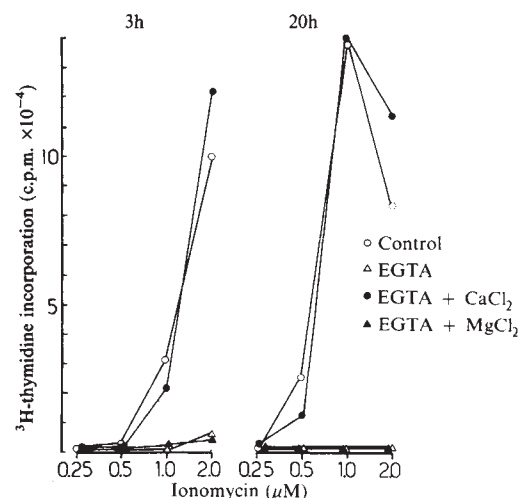


Fig. 2 Inhibition by EGTA of ionomycin- and TPA-induced ^3H -thymidine incorporation into T lymphocytes and reversibility with excess CaCl_2 . T lymphocytes were placed in 96-well microtitre plates containing culture medium supplemented or not with 2 mM EGTA alone, EGTA plus 2 mM CaCl_2 or EGTA plus 2 mM MgCl_2 . In all cases the pH of the medium was pre-adjusted to 7.4. The cells were stimulated with different concentrations of ionomycin and 10 nM TPA. After 3 or 20 h incubation (37°C) the supernatant was removed and replaced with fresh culture medium. ^3H -thymidine incorporation was measured as described in Fig. 1 legend.

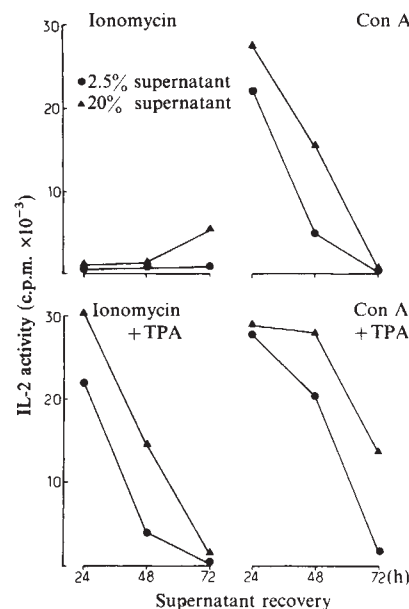


Fig. 3 IL-2 secretion by T lymphocytes after stimulation by ionomycin and TPA or Con A. T lymphocytes were stimulated by ionomycin (1 μM) or Con A (5 $\mu\text{g ml}^{-1}$) with or without TPA (10 nM) in Costar wells at 4×10^6 cells per well (4 wells per group). After 3 h, they were centrifuged, the supernatant carefully aspirated and replaced with 1 ml of fresh culture medium (see also Fig. 1 legend), and 24, 48 or 72 h later, 200 μl of supernatant were removed from each group and stored (4°C) until tested. To test for IL-2 activity, 10^4 cells from an IL-2-dependent T-lymphocyte clone, CTL-L¹⁵, were dispersed into 96-well microtitre plates and supernatant added to a final concentration (% volume) of 2.5 or 20%. After 18 h incubation (37°C), all wells were pulsed with 0.5 μCi of ^3H -thymidine and cells were collected 6 h later for β counting.

IL-2 activity decreased markedly. Con A was more effective at causing IL-2 secretion when combined with TPA than alone, because supernatants obtained 48 and 72 h after stimulation of cells with Con A plus TPA contained greater IL-2 activity than those obtained at the same time from cells stimulated with Con A alone.

Table 1 Incorporation of ^3H -thymidine by CTL-L cells in the presence of mitogenic agents or interleukin-2

	Ionomycin				Con A 5 $\mu\text{g ml}^{-1}$	IL-2					Control
	2 μM	1 μM	0.5 μM	0.25 μM		5%	1.6%	0.55%	0.18%	0.06%	
No TPA	620	803	1,029	745	901	26,365	25,573	18,945	9,391	3,871	316
TPA 1.5 nM	479	289	357	389	446	ND	ND	ND	ND	ND	593
3 nM	1,152	1,500	876	720	769	ND	ND	ND	ND	ND	727
10 nM	5,940	4,162	4,415	4,557	2,638	ND	ND	ND	ND	ND	3,053

10^4 CTL-L cells were dispersed into 96-well microtitre plates. Ionomycin, Con A, TPA or supernatant from IL-2-secreting EL4 cells¹⁶ were added to give the desired final concentration. After 18 h incubation (37 °C), all wells were pulsed with ^3H -thymidine and cells collected 6 h later for β counting. ND, not determined.

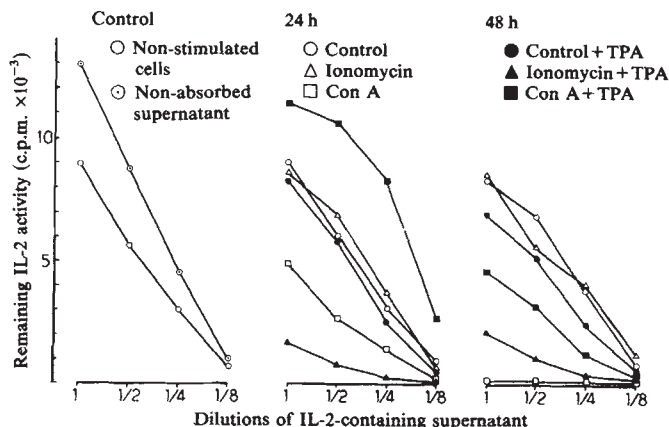


Fig. 4 Expression of the receptor for IL-2 by T lymphocytes after stimulation by ionomycin and TPA or Con A. Measurement was done by absorbing IL-2 from standard IL-2-containing supernatant by the stimulated T cells and testing for residual activity on CTL-L cells. T lymphocytes were stimulated with ionomycin (1 μM) or Con A (5 $\mu\text{g ml}^{-1}$) with or without TPA (10 nM) in Costar wells at 5×10^6 cells per well. After 3 h, they were centrifuged, the supernatant carefully aspirated and replaced with 1 ml of fresh culture medium (see also Figs 1 and 3 legends), and 24 or 48 h later, the cells were recovered and incubated (37 °C) for 1 h in fresh culture medium; 10^7 cells were then pelleted and incubated (4 °C) with supernatant (2%) from an IL-2-secreting EL4 cell line¹⁶ for 1 h with intermittent agitation. The supernatant was recovered and tested for activity on CTL-L cells as described in Fig. 3 legend. 10^4 CTL-L cells (75 μl) cultured for 18 h with 50 μl of absorbed supernatant (diluted or non-diluted) were pulsed with ^3H -thymidine and collected 6 h later for β counting. The control figure represents the activity of the non-absorbed supernatant and that absorbed by non-stimulated cells.

T lymphocytes stimulated by ionomycin and TPA were also induced to express the receptor for IL-2 (Fig. 4). At 24 or 48 h after treatment with the various mitogenic agents or medium alone, the cells were recovered, washed and allowed to absorb at 4 °C supernatant from an EL4 cell line that secretes IL-2¹⁶. The absorbed supernatant was then tested for activity on CTL-L cells. Figure 4 shows that T lymphocytes can absorb IL-2 activity 24 and 48 h after stimulation with ionomycin and TPA, whereas ionomycin or TPA alone have no effect. Also, T lymphocytes activated by Con A similarly absorb IL-2 activity although those activated by Con A in the presence of TPA do not. We believe this to be due to an enhanced stimulation of IL-2 secretion by Con A and TPA (see Fig. 3) from IL-2-secreting subpopulations, resulting in the saturation of the receptor by its ligand.

The expression of the receptor for IL-2 is an obligatory step for the induction of T-lymphocyte proliferation¹⁷. Note that, although synergy between A23187 and TPA has previously been intimated¹⁸, these agents are not in themselves mitogenic, as they do not abrogate the requirement for IL-2 which alone drives mitogenesis. Thus, a T-cell clone, CTL-L, which constitutively expresses the receptor for IL-2 and requires exogenous IL-2 for growth, does not proliferate in response to ionophore and TPA at concentrations that are normally mitogenic (Table 1). Furthermore, work now in progress (A.T. *et al.*, unpublished) shows that cytotoxic T lymphocytes (Lyt 2⁺, T4⁺), which themselves

do not secrete sufficient IL-2 for proliferation, when purified from spleen cells of preimmunized mice, are refractive to the mitogenic effect of ionomycin plus TPA unless exogenous IL-2 is added to the culture; they then also acquire the capacity to kill specific target cells. Therefore, the calcium ionophores, in conjunction with TPA, mimic the effect of antigen or Con A but not that of IL-2, that is, although they can bypass the initial stimulus leading to the production of IL-2 and expression of IL-2 receptors, they cannot bypass the later step of activation resulting from the engagement of IL-2 and its receptor. Our results provide a way of experimentally dissecting the early steps of lymphocyte activation. More generally, they are consistent with the possibility that antigenic activation of T lymphocytes proceeds via the production of the putative second messengers 1,2-diacylglycerol and inositol triphosphate, as has been suggested for other systems⁵⁻⁸.

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Control of haemoglobin switching by a developmental clock?

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The pattern of haemoglobin production changes at the embryonic, fetal and postnatal stages of human development, reflecting the expression of different globin genes in both the α -like and β -like gene clusters^{1,2}. Recent studies have identified alterations in the state of DNA methylation³ and sensitivity to nuclease digestion⁴

associated with developmental expression of the globin genes in red blood cell precursors, but the mechanism initiating these changes remains unknown. Despite the screening of large numbers of blood samples from newborn infants^{5,6}, no mutants have been found which affect the timing of these changes (with one possible exception involving a chromosomal translocation⁷), thus necessitating alternative approaches to analysing the cellular basis for the timing of haemoglobin switching. Although many mechanisms are possible, the initiation of the switch from fetal to adult haemoglobin could be regulated essentially either by a developmental clock inherent to haematopoietic stem cells or by an inductive environment^{8,9}, and in an attempt to distinguish between these possibilities, we have transplanted sheep fetal haematopoietic tissue into adult animals. Although previous experiments of this type produced conflicting results¹⁰⁻¹², the accumulated results presented here demonstrate that the pattern of haemoglobin production after transplantation is determined largely by the gestational age of the fetal donor cells.

The cellular basis of haemoglobin switching may involve a change of gene expression within a single erythroid (haematopoietic) lineage or the replacement of one lineage (committed to a fetal red cell programme) by another (committed to the adult pattern)¹³; however, there is considerable evidence to refute the latter model¹⁴⁻¹⁹. Thus, given that switching involves a change in gene expression within the same lineage, three different patterns of haemoglobin synthesis might be observed after transplanting fetal haematopoietic tissue into an adult animal^{11,12}. If switching from fetal to adult haemoglobin is mediated by a signal present for a short time at the end of gestation, the transplanted fetal cells should continue to produce fetal haemoglobin indefinitely because exposure to the signal would be bypassed by transplantation. If, on the other hand, the type of haemoglobin produced is regulated by the 'adult environment' of the haematopoietic tissue, the transplanted cells should be induced to synthesize adult haemoglobin, regardless of their gestational age. Finally, if switching is regulated by a developmental clock inherent to haematopoietic stem cells, the adult environment may have only a permissive role, and switching from fetal to adult haemoglobin production should occur in the transplanted cells at a time closely related to gestational age.

Details of the transplantation procedure have been described previously¹¹. Briefly, recipient sheep, 3-12 months old and homozygous for the $\Lambda\beta$ type of adult haemoglobin, were given total body irradiation as either a 12-Gy single dose or an 18-Gy divided dose given in two or three equal fractions, 48 h apart. Donor fetuses, homozygous for the β type of adult haemoglobin, from timed matings (accurate to 1 day) were obtained by caesarian section, and haematopoietic cells were prepared from fetal liver (after depletion of hepatocytes), bone marrow and thymus. These cells were slowly transfused into the recipient via an indwelling catheter within 4 h of the final irradiation dose. The transplanted animal was then supported with fluids, antibiotics and irradiated platelet transfusions. Blood and bone marrow samples were obtained regularly to assess the degree of haematopoietic recovery, for chromosome studies and to measure the relative rates of fetal and adult haemoglobin synthesis. Transplanted animals survived only for up to 41 days, even though we varied pre-transplant preparation and increased

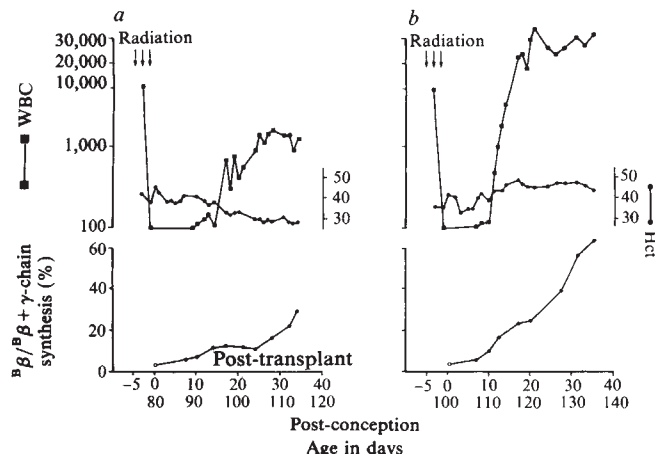


Fig. 1 Haematological and globin synthesis data on two representative animals transplanted with haematopoietic cells from fetuses of gestational age 80 days (a) and 100 days (b). Blood samples were obtained regularly from an indwelling catheter for haematocrit (Hct) and white blood cell (WBC) counts, while bone marrow samples were obtained twice weekly for morphological examination and measurements of globin-chain synthesis. Washed marrow cells were incubated with 50 μ Ci 3 H-leucine for 60 min and protein precipitated by acid-acetone. Partial purification of globin was obtained by gel filtration on Sephadex G-100 in 5% HCOOH and individual globin chains separated by CM-cellulose chromatography in 8 M urea using a 0.0046-0.031 M Na_2HPO_4 gradient at pH 6.6 (see ref. 11). Globin synthesis results are expressed as the proportion of donor β -chain synthesis over the total donor non- α -chain synthesis ($\beta/\beta + \gamma$). Recipient $\Lambda\beta$ ($+\beta$)-chain synthesis was minimal or undetectable.

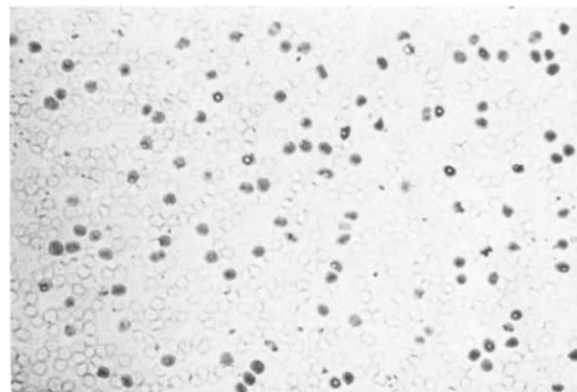


Fig. 2 Peripheral blood film from the sheep in Fig. 1a, 34 days after transplantation with 80-day fetal liver cells. The acid elution technique²³ preferentially removes adult haemoglobin, distinguishing cells containing largely fetal haemoglobin (darkly stained) from the adult (ghost) cells.

the intensity of post-transplantation support. There was no evidence of graft-versus-host disease.

Previous control studies¹¹ have demonstrated that in these conditions no autologous marrow recovery occurs and there is insignificant recipient haemoglobin synthesis (marked by $\Lambda\beta$ chain production). Furthermore, in adult to adult marrow transplantations, no reactivation of fetal haemoglobin is observed.

Despite the relatively short survival time of the recipient animals, the post-transplantation patterns of haemoglobin synthesis by the donor cells showed clear differences. Figure 1 illustrates the pattern of haematopoietic recovery and haemoglobin synthesis in two representative experiments. Initially, donor cells from an 80-day-gestation fetus synthesized mainly fetal haemoglobin, and by 34 days, fetal-haemoglobin-containing cells made up ~25% of the total circulating red cells (Fig. 2). At 24 days after transplantation, the γ -chains of HbF still account for ~90% of the total $\gamma + \beta$ -chain production, but

Table 1 Proportions of donor and recipient dividing haematopoietic cells (direct) and 'marrow stromal' cells (after culture) in bone marrow samples from two transplanted animals

			Chromosome analysis (donor/recipient karyotypes)	
Post- conception	Days Post- transplant	% γ -chain synthesis	Direct	After culture (days)
104	24	89	100/0	13/87 (24)
132	32	44	98/0	5/42 (16)
				15/120 (37)

In both cases, the donor fetus was male and the recipient female.

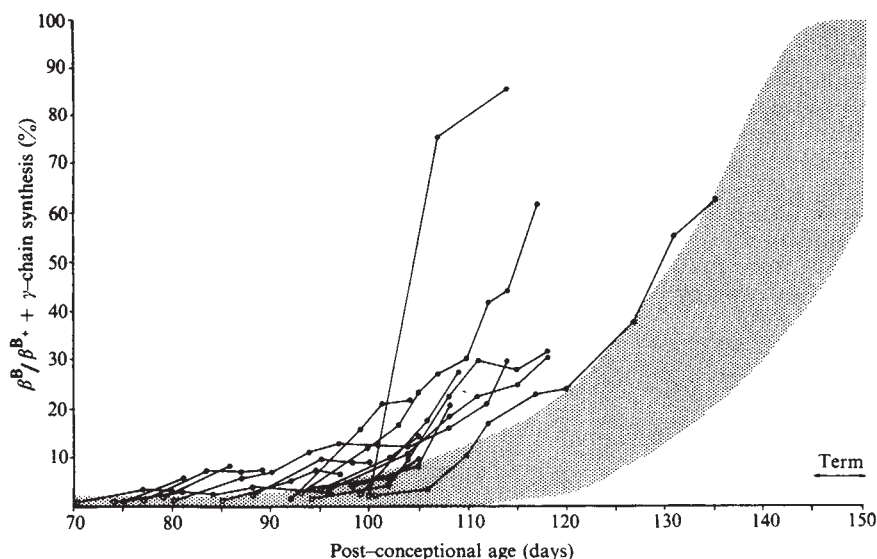


Fig. 3 Combined globin synthesis data on 18 transplantation experiments carried out with fetal cells at 70–105 days' gestation. Only those experiments with good evidence of engraftment (based on bone marrow cellularity and recovery of peripheral blood white blood cells) and survival > 14 days have been included. The shaded area represents the normal range of $\beta/\beta + \gamma$ -chain synthesis in peripheral blood reticulocytes obtained from >20 chronically catheterized normal sheep fetuses (ref. 24 and our unpublished observations). Open circles are the results from donor reticulocytes at the time of transplantation; closed circles are results from bone marrow samples from the transplanted recipient.

thereafter, β -chain synthesis begins to increase sharply (Fig. 1a). In contrast, similar studies on a 100-day-gestation donor fetus (Fig. 1b) showed an almost immediate increase in β -chain production after transplantation, rising to 65% by 35 days post-transplantation. Clearly, the time of haemoglobin switching after transplantation differs in these two cases; because, in both cases, the increase in β -chain synthesis occurred at a post-conceptual age of 105–110 days, this may be related to the gestational age of the cells.

Figure 3 shows the combined globin synthesis data from 18 animals, transplanted with fetal cells from donors of 70–105 days' gestation, plotted against the post-conceptual age of the cells. Clearly, the pattern observed in the two representative cases illustrated above holds true for the combined data. Little or no increase in donor β -chain synthesis is observed until the cells are ~100 days post-conception, irrespective of age at transplantation. Thereafter, there is a rise in β -chain synthesis which parallels but precedes that observed in reticulocytes from normal fetuses sampled *in utero*.

It is possible that the pattern of switching in the haematopoietic cells was being modified by accessory cells transplanted at the same time. Indeed, recent work suggests that in some human marrow transplant recipients, marrow stromal cells are of donor origin²⁰. To exclude this possibility, bone marrow samples were cultured at various times after transplantation in conditions used for long-term human marrow culture²¹. A stromal layer developed in the flasks, similar to that seen in murine and human marrow culture. When haematopoiesis had substantially decreased (normally after 2–3 weeks), the remaining stromal cells were karyotyped to determine whether they were of donor or recipient origin, based on sex chromosome differences. The results (Table 1) show that in the two animals illustrated in Fig. 1, bone marrow samples taken 24 and 32 days after transplant gave 100% donor karyotypes on direct chromosome analysis (the dividing haematopoietic cells), but the stromal layers derived from these marrows were largely of recipient origin. Thus, at a time when the transplanted erythroid cells were producing mainly fetal haemoglobin, they were maturing among largely adult stromal cells. It is impossible to determine whether the few donor-type metaphases seen among these cells were haematopoietic cells persisting in the culture or donor stromal cells. Thus, although not conclusive, this experiment suggests that a putative developmental clock is more likely to reside within the haematopoietic cells themselves.

These results indicate that the pattern of haemoglobin synthesis in fetal cells after transplantation to an irradiated adult seems to be determined more by their gestational age than by the length of time for which they develop in an adult milieu. This suggests that the developmental pattern of haemoglobin

synthesis is programmed in haematopoietic stem cells during early fetal life. However, the overall pattern of haemoglobin switching in the transplanted fetal cells is slightly advanced compared with that of the normal developmental switching process, although this may not be surprising considering that the transplant model is somewhat removed from the normal physiological process. It is possible that the adult environment provides an essential permissive factor that does not itself appear in normal development until ~120 days' gestation, when the switch occurs *in utero*. The appearance on the fetal cells, at about 100–110 days' gestation, of a receptor capable of interacting with this 'factor' could explain the gestational age effect.

Alternatively, the slight but consistent advancement of switching in these experimental conditions may provide a clue to the nature of the developmental timing mechanism. Repopulation of the adult marrow by the fetal cells presumably requires a marked increase in the rate of division of the donor stem cells, of which, at least in other species, only a small proportion are normally in cycle at any one time. If increased stem cell division were responsible for the advancement in the time of switching in the experimental animals, it might implicate the number of stem cell divisions as the basis for the developmental clock. Recent studies²² showing a precocious switch from larval to adult haemoglobin production in anaemic amphibians, unrelated to metamorphosis, supports this possibility.

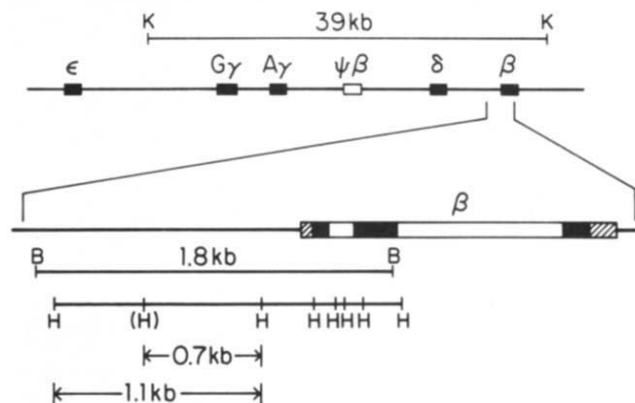
Thus, allowing for perturbations due to marrow transplantation, our experiments suggest that the timing of the switch from fetal to adult haemoglobin in ruminants at least in part reflects inherent programming of the haematopoietic stem cells. The notion that the activation of this programme may be mediated through the number of stem cell divisions has the attraction that it is open to experimental testing.

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G to A substitution in the distal CCAAT box of the γ -globin gene in Greek hereditary persistence of fetal haemoglobin

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The sequence 5' TTGGPyCAAT 3' (the 'CCAAT box') is a constituent of the promoter region of many eukaryotic and prokaryotic genes^{1,2} and is believed to play a part in promoter function^{3,4}. A characteristic of the two fetal human globin genes (γ and δ) is a duplication of a 12-base pair (bp) sequence containing the CCAAT box. Here we report a G $\rightarrow$ A substitution in the TTG sequence of the distal CCAAT box of the γ -globin gene in an individual with the γ (Greek) type of hereditary persistence of fetal haemoglobin (HPFH). This represents the first report of a natural mutation of the CCAAT box in a eukaryotic gene. The fact that this transition is associated with inappropriate expression of the γ gene in adult life suggests that the CCAAT box (or its surrounding sequences) may have a role in the developmental control of γ -globin genes.

In the human, the β -globin genomic region contains five genes arranged in the order of their expression during ontogeny: ϵ , γ , δ and β (reviewed in refs 5-7). Both γ and δ genes are expressed in fetal life and in the traces of fetal haemoglobin found in the normal adult⁷. In HPFH, however, high levels of fetal haemoglobin are found in adults who are otherwise haematologically normal⁷. In the common African variants of HPFH, both γ and δ chains are produced as a result of large deletions that remove the δ and β genes and leave the γ and δ genes intact⁷⁻¹⁰. In the Greek type of HPFH¹¹ there is continued γ chain synthesis in the adult^{7,12-14} in the absence of globin gene anomalies detectable by Southern blotting^{9,10,15,16}. We searched for the underlying mutation by sequencing the γ genes of a Greek γ HPFH chromosome.

As Greek γ HPFH is found only in heterozygotes, restriction site polymorphisms in the globin cluster were used to distinguish between the HPFH and the non-HPFH chromosomes. A female compound heterozygote for γ HPFH and β -thalassaemia had seven children, three with HPFH and four with heterozygous β -thalassaemia¹³. This patient was also heterozygous (+/-) for the *HinfI* site^{17,18} located ~930 bp 5' to the β gene (Fig. 1). Her husband was homozygous (+/+), and their three HPFH offspring were homozygous (+/+), while their four β -thalassaemia offspring were heterozygous (+/-), indicating that the *HinfI* polymorphic site is present on the HPFH chromosome of the

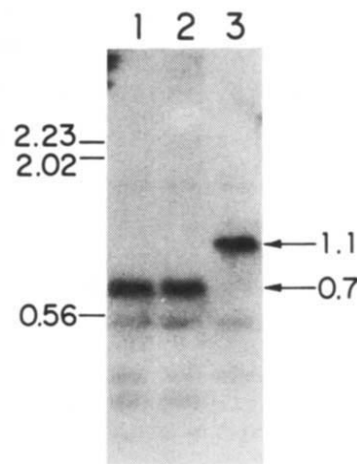


Fig. 1 Cloning of the cosmid carrying the HPFH determinant. Three cosmids which carried the 39-kb *KpnI* fragment (see below) were examined for the presence of the polymorphic *HinfI* site located 0.9 kb 5' to the β gene. DNA (0.5 μ g) from each of cosmids cES2, cES3 and cES13 was digested with *HinfI*, fractionated on a 1.4% agarose gel, transferred to nitrocellulose²⁶, and hybridized to a probe labelled with [α -³²P]dCTP and [α -³²P]dTTP by nick-translation. The probe was a 1.8-kb *BamHI* fragment prepared from λ HBG1 (ref. 27) which carries the 5' portion of the β gene and 1.5 kb of upstream sequences as shown. K, B and H represent cleavage sites for *KpnI*, *BamHI* and *HinfI*, respectively. The polymorphic *HinfI* site is shown in parentheses. The *HinfI* sites which lie within the β gene are from ref. 28. The presence of the *HinfI* site results in the detection of a 0.7-kb fragment (as well as several smaller fragments) on genomic hybridization experiments; lack of the site results in the detection of a 1.1-kb fragment. The *HinfI* fragments of lower relative molecular mass are not retained efficiently in these filter hybridization experiments. On the map of the gene cluster, expressed genes are shown as solid boxes and the pseudo gene is shown as an open box. For the β gene, untranslated regions, exons, or introns are represented by hatched, solid and open areas, respectively.

Methods. DNA was isolated²⁹ from peripheral blood lymphocytes of a Greek HPFH/ β -thalassaemia compound heterozygote, and was digested to completion with *KpnI* as judged by the appearance of characteristic satellite bands derived from the *KpnI* repeat family on agarose gel electrophoresis³⁰. The 35-45-kb fraction was isolated from 5-20% NaCl velocity sedimentation gradients prepared in 0.01 M Tris-HCl pH 7.9, 2 mM Na₂EDTA. Gradients were centrifuged for 5½ hours at 35,000 r.p.m., 19°C, in the SW41 rotor. Cosmid vector arms were prepared from plasmid pHC79 (ref. 31) in which the *BamHI* site had been adapted to accept *KpnI* target fragments (R.G., unpublished). Vector arms having *EcoRI*-*KpnI* or *KpnI*-*Sall* termini were ligated to target DNA at a 2:1 molar ratio following a strategy designed to prevent formation of vector concatemers³². Over 5×10^6 colonies were obtained per 0.1 μ g of size-selected human DNA. General conditions for ligation, packaging, infection, plating, library screening, colony isolation, and preparation of cosmid DNA have been described elsewhere^{33,34}. About 120,000 unamplified colonies were screened with probes derived from the γ and δ genes.

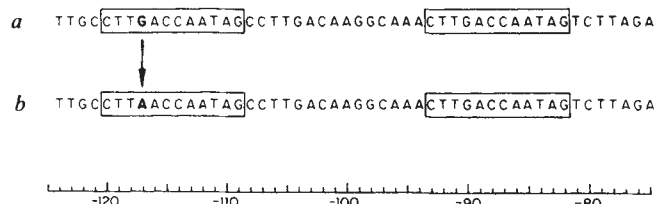


Fig. 2 *a*, Sequence of the normal $\alpha\gamma$ gene. *b*, Sequence of the $\alpha\gamma$ gene from the $\alpha\gamma$ HPFH chromosome. Nucleotides -75 to -124 (relative to the cap site) are shown. The 12-bp direct repeat sequences, which include the CCAAT boxes, are enclosed. The G to A transition at position -117 is marked by an arrow.

Methods. The 2.5-kb *Bam*HI fragment from cES2 which carries 2 kb of 5' flanking region and the first two exons of the $\alpha\gamma$ gene, and the 2.6-kb *Eco*RI fragment from cES2 which carries most of the $\alpha\gamma$ gene and 1,200 bp of 5' flanking sequence, were subcloned into M13mp18 (ref. 35). A set of deletions was generated with exonuclease III and *S*₁ nuclease to facilitate sequence analysis³⁶, and DNA sequences were determined by the chain termination method of Sanger³⁷. Sequence data were derived for each strand independently in the promoter regions (residues -160 to +28) and from one strand in the other regions of the fragment. In the latter regions, most of the data were determined on two or more separate templates, sequencing reactions and autoradiograms.

patient but absent from the thalassaemia chromosome. As the *Hinf*I site that distinguishes the two chromosomes is located ~20 kilobase pairs (kb) 3' to the γ genes, construction of cosmids was required to generate clones containing both the γ genes and the polymorphic site.

To retrieve a clone which included $\alpha\gamma$, δ and β genes on one DNA fragment, we took advantage of the fact that *Kpn*I cleaves outside the $\alpha\gamma$ - to β -gene region, yielding a fragment of 39 kb^{19,23}. The *Kpn*I map of the proband (Fig. 1) was confirmed by double digestion experiments which supported the suitability of this strategy. Using proband DNA of high relative molecular mass, a cosmid library was prepared as described in Fig. 1 legend. Approximately 120,000 colonies were screened. Natural *Eco*RI fragments of 2.6 kb and 2.25 kb which carry, respectively, portions of the $\alpha\gamma$ and δ genes, were labelled and used as probes for screening. Four positive colonies were isolated, three of which (cES2, cES3 and cES13) were studied in detail. cES2 and cES3 contained the *Hinf*I site linked to the HPFH phenotype; cES13 lacked this site and thus contained the thalassaemia locus (Fig. 1).

The $\alpha\gamma$ and $\alpha\gamma$ genes of the HPFH chromosome were isolated from cES2 and sequenced, as was the $\alpha\gamma$ gene from the non-HPFH chromosome (cosmid cES13). The data were compared base-by-base with the previously determined sequences (refs 20, 21 and J. Slightom, personal communication) of the γ -globin genes. Two variant sequences of γ genes, defined as chromosomes A and B, have been described (ref. 20 and J. Slightom, personal communication). Both the $\alpha\gamma$ and $\alpha\gamma$ genes from cES2 and the $\alpha\gamma$ gene from cES13 were of the B-chromosome type.

We sequenced 2,600 bp of the $\alpha\gamma$ gene of the HPFH chromosome, from -2,122 to +478; only one difference compared with the normal $\alpha\gamma$ gene was noted, a T $\rightarrow$ C substitution at position -158. The normal $\alpha\gamma$ and $\alpha\gamma$ genes also differ from each other by a C $\rightarrow$ T transition at this position²⁰, suggesting that either pyrimidine will suffice for normal regulation of expression.

We found two base differences in the 2,648 residues of the $\alpha\gamma$ gene sequence (-1,220 to +1,420) from the HPFH chromosome: at -831 the $\alpha\gamma$ HPFH gene differs from the $\alpha\gamma$ gene of chromosome B by an extra A (an A in this position is the feature of chromosome A²¹) and at position -117, where the normal $\alpha\gamma$ gene has a G, the $\alpha\gamma$ gene from the HPFH chromosome has an A residue on the sense (or message) strand; this site lies within the distal CCAAT box of the $\alpha\gamma$ gene (Fig. 2). The sequence of the promoter region (-150 to +28) of the $\alpha\gamma$ gene

from the thalassaemia chromosome of the proband is identical to the B-type chromosome. Thus, the $\alpha\gamma$ (Greek) HPFH chromosome studied carries a G to A transition at position -117 in the promoter region of the $\alpha\gamma$ gene. An identical point mutation has been found by Collins *et al.*²² in an unrelated $\alpha\gamma$ HPFH individual.

The functional consequences of the substitution reported here will remain speculative until efficient *in vitro* systems allowing analysis of the developmental control of the γ -globin genes in erythroid cells become available. It is conceivable that our finding reflects a chance association of a DNA polymorphic site with the $\alpha\gamma$ HPFH phenotype. It is more likely, however, that there is a causative relationship between the substitution at position -117 of the $\alpha\gamma$ gene and the $\alpha\gamma$ HPFH phenotype; in support of this notion, transient expression assays suggest that the CCAAT box has a role in the control of globin gene transcription^{3,4}. It has also been shown that while single base mutations of residues -115 to -112 of the CCAAT box of the mouse β -major gene decrease transcription, mutations of residues -117 and -116 increase enhancer-dependent transcription in transient assays (T. Maniatis, personal communication).

Observations in non-deletion HPFH mutants may allow detection of those elements of the globin genomic region that have a role in the developmental control of globin genes. Other studies have revealed substitutions at positions -202 or -196 of the $\alpha\gamma$ or $\alpha\gamma$ genes in individuals who have inappropriate expression of these genes^{23,24}. Our results suggest that the CCAAT box is one of the elements which contribute to the developmental control of γ -globin expression.

We speculate that in adult erythroid cells, the CCAAT box region of the γ genes is the binding site of a repressor; this results in the 'turning-off' of the γ genes in the normal adult. A G $\rightarrow$ A substitution at -117 of the distal CCAAT box of the $\alpha\gamma$ gene may inhibit the DNA-protein interaction, hence allowing the inappropriate expression of that gene in Greek $\alpha\gamma$ HPFH. Alternatively, expression of the γ genes may depend on binding of a positive *trans*-acting factor normally present in fetal cells. The substitution in Greek $\alpha\gamma$ HPFH may remove the requirement for this interaction, enabling RNA polymerase to initiate at this site. This mutation would thus be analogous to the *lacUV5* mutation in *Escherichia coli* in which the requirement for the positively acting cap protein is lost²⁵.

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A point mutation in the $\alpha\gamma$ -globin gene promoter in Greek hereditary persistence of fetal haemoglobin

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Hereditary persistence of fetal haemoglobin (HPFH) is a benign condition characterized by the production in adulthood of more than 1% fetal haemoglobin (HbF, $\alpha_2\gamma_2$) in the absence of erythropoietic stress¹. Several genetic types have been discerned based on the level of HbF produced, the relative contributions of the duplicated fetal (γ and $\alpha\gamma$) globin genes, and the presence or absence of deletions involving the β and δ genes in *cis* to the mutation^{2,3}. Greek HPFH is a non-deletion variety in which heterozygotes produce 10-20% HbF, predominantly due to overproduction of the $\alpha\gamma$ chain⁴⁻⁹. We have cloned a 40-kilobase (kb) region of the β -globin cluster from a Greek HPFH allele and report here that a point mutation (G $\rightarrow$ A) occurs 117 base pairs (bp) 5' to the cap site of the $\alpha\gamma$ -globin gene, just upstream of the distal CCAAT sequence. The corresponding region of the γ -globin gene is normal. We discuss the implications of this finding for the developmental regulation of globin gene expression.

No homozygotes for Greek HPFH have been described. The individual studied here (I-3 in Fig. 1) is doubly heterozygous for Greek HPFH and β -thalassaemia and, as the pedigree indicates, the HPFH and β -thalassaemia mutations are present in *trans*. In order to unequivocally define alleles in cloned DNA from such an individual, we used an approach described previously¹⁰ to obtain cosmid clones bearing the 40-kb *KpnI* fragment which extends from 6 kb 5' to the γ gene to 3 kb 3' to the β gene, thereby including γ , $\alpha\gamma$, $\psi\beta$, δ and β -globin genes¹⁰. Seven cosmids were obtained, and no restriction site differences were found in digests with more than 20 enzymes. Both the HPFH and β -thalassaemia alleles were haplotype I at the polymorphic restriction sites described by Orkin, Kazazian and co-workers^{11,12}.

The 5.0-kb *BglII* fragments containing the β -globin genes were subcloned from the seven cosmids into the transient expression vector pLTN3, and transfected into monkey kidney (COS) cells¹³. For three of the cosmids *S*₁ nuclease analysis¹⁴ and DNA sequencing revealed a previously undescribed point mutation at position 116 of intervening sequence 1 (IVS1) which creates an abnormal acceptor site and thus results in β thalassaemia (F.S.C., J.E.M., J.P., S.M.W. and B.G.F., in preparation). The other four β genes produced normal amounts of normally spliced β -globin mRNA (Fig. 2) and had normal β IVS1 sequences, thus these four cosmids were derived from the Greek HPFH allele.

Because the overproduction of HbF in this condition is primarily due to $\alpha\gamma$ globin, we focused our sequencing efforts

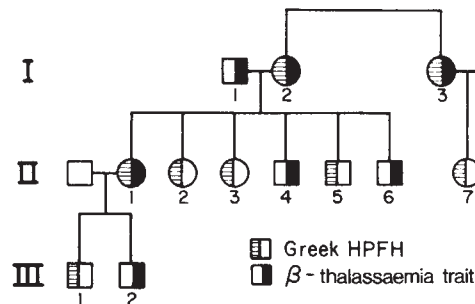


Fig. 1 Pedigree of the Greek HPFH family, originally described in ref. 4. Individual I-3, who is doubly heterozygous for Greek HPFH and β thalassaemia, was the source of DNA for cosmid cloning.

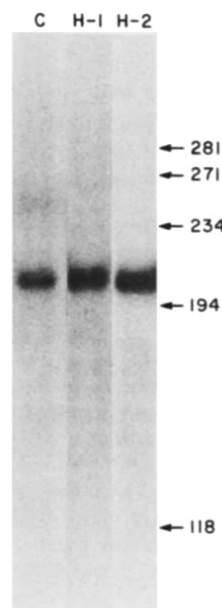


Fig. 2 *S*₁ nuclease analysis of β -globin mRNA transcripts from a normal β -globin gene (C) and two representative clones derived from the Greek HPFH β -globin gene (H-1 and H-2). The 5.0-kb *BglII* β fragments were cloned into the SV40 enhancer-containing expression vector pLTN3 (ref. 13). Transfection of monkey kidney COS cells, preparation of cytoplasmic mRNA and *S*₁ nuclease analysis were performed essentially as described previously²⁸. The *S*₁ probe was a 1.9-kb *BamHI* genomic β fragment labelled at its 5' end, which gives rise to a 209-bp fragment (corresponding to exon 2) when hybridized with β -globin mRNA²⁸. The amount of β -globin mRNA produced from the Greek HPFH β genes (H-1 and H-2) is not reduced relative to that produced from a control gene (C), even though *in vivo* there is probably reduced β -globin synthesis in heterozygotes for Greek HPFH². As a control for transfection efficiency, nuclear DNA was also prepared from transfected cells and dot-blotted²⁹ with a β probe (the purified 4.4-kb *PstI* genomic fragment); the amounts of β -specific nuclear DNA were essentially the same in all three samples (data not shown).

on the $\alpha\gamma$ promoter region. The 7.5-kb γ and 3.5-kb $\alpha\gamma$ *HindIII* fragments were subcloned into plasmid pBR322, then further subcloned into the phage M13mp8 and sequenced by the dideoxy method¹⁵. The γ gene from -141 to +289 (relative to the cap site) had a sequence identical to that of the normal 'chromosome B' allele (refs 16, 17 and J. Slightom, personal communication). The $\alpha\gamma$ gene, sequenced from -486 to +578, was identical to chromosome B except for a G $\rightarrow$ A point difference at -117, just two bases 5' to the distal CCAAT sequence (Fig. 3). Another individual with Greek HPFH not known to be related to our patient has been found to carry the same point mutation¹⁸.

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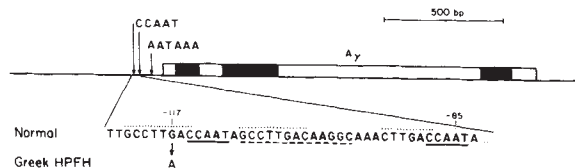


Fig. 3 A point mutation in the $\text{A}\gamma$ -globin gene promoter (117 bp 5' to the cap site) in Greek HPFH. The G $\rightarrow$ A change occurs just 5' to the distal CCAAT box. Both CCAAT sequences are underlined. The point mutation occurs in an 8-bp sequence (marked by dotted lines above the sequence) that is repeated on either side of the distal CCAAT. The last 6 bp of this are repeated again just before the proximal CCAAT, as part of the longer duplicated segment which results in the pair of CCAAT regions. There is also a 13-bp perfect palindrome (dashed line) between the CCAAT boxes, the significance of which is unknown.

The identification of this point mutation does not, of course, prove that it is the cause of the $\text{A}\gamma$ -globin overproduction in Greek HPFH; the possibility that this represents a polymorphic difference with no functional consequences cannot be excluded at present. However, a comparison of globin gene promoters^{3,19} shows that the sequence YYTTGA (where Y = pyrimidine) immediately precedes the CCAAT box in all functioning descendants of the 'proto- ϵ ' and 'proto- γ ' genes²⁰ in man, goat, rabbit and mouse. The adult β -globin genes usually have a G residue just 5' to CCAAT, but in adult genes this region appears otherwise unconstrained. Conservation of this six-base sequence in embryonic and fetal genes over ~200 Myr of evolutionary time^{19,20} implies functional significance.

Superficially, it seems surprising that the -117 mutation does not result in reduced, rather than increased, expression of the $\text{A}\gamma$ -globin gene. Analysis of the rabbit β -globin gene promoter by sequential deletions and site-directed mutagenesis has shown that deletions or single base changes involving the CCAAT sequence itself result in decreased transcriptional efficiency of this gene when introduced in a polyoma or simian virus 40 (SV40) viral vector into mouse or human non-erythroid cells^{21,22}. Similarly, mutations within the CCAAT sequence of the mouse β -major gene reduce its expression in HeLa cells. Remarkably, however, a G to A mutation 2 bp 5' to the CCAAT box (a base change identical to that reported here in the human $\text{A}\gamma$ gene) results in a twofold increase in transcription of the mouse β -major gene (R. Myers and T. Maniatis, personal communication). It is possible, therefore, that the -117 mutation in the $\text{A}\gamma$ -globin gene increases the efficiency of the promoter element in adult erythroid cells.

Note, however, that the CCAAT region is not always an essential positive regulatory element; deletion of the CCAAT region of the sea urchin *H2A* gene increases its expression in

Xenopus oocytes²³, and some promoters, most notably that of the well-studied herpes simplex thymidine kinase gene, lack a CCAAT element altogether²⁴. It is conceivable that in some situations the CCAAT region is involved in differential control of gene expression. The -117 $\text{A}\gamma$ -gene mutation could reduce the effectiveness of this region as a normally negative promoter element in adult erythroid cells. Alternatively, the two CCAAT boxes may be involved in competitive inhibition, so that inactivation of the distal one leads to more effective use of the proximal one. In fact, removal of all or part of one of the two CCAAT sequences results in a 1.5 to 4 fold increase in expression of γ -globin genes in gene transfer experiments using HeLa cells³⁰.

At least two characteristics of the Greek HPFH phenotype remain unexplained: (1) while the β -globin genes from the Greek HPFH cosmids function normally in a transient expression system (Fig. 2), there is evidence that *in vivo* β -globin gene expression is moderately reduced in *cis* to the HPFH mutation², implying some sort of 'cross-talk' between γ and β genes, perhaps involving unknown mechanisms regulating the maximum total output of the non- α -globin genes from a given chromosome. This reciprocal relationship between γ - and β -globin accumulation also occurs *in vivo* during the normal fetal to adult globin switch²⁵ and in F cells in normal adults, which have proportionately less β globin than non-F cells³¹. (2) There is reasonably good evidence *in vivo*, and considerable evidence from cultured colonies derived from erythroid burst-forming units (BFU-E)⁸, that the $\text{G}\gamma$ -globin gene (presumably in *cis*, though a *trans* effect has not been excluded) is also moderately overexpressed in Greek HPFH. This could be attributed possibly to proximity to the mutant $\text{A}\gamma$ gene; in somatic cells co-transfected with a globin gene and a thymidine kinase gene, for example, co-expression and co-repression occur even though the genes are up to 20 kb apart²⁶.

Recently, we have postulated the existence of regulatory sequences in the -200 region of the human γ -globin genes, based on a point mutation found at position -202 of the overexpressed $\text{G}\gamma$ gene in $\text{G}\gamma\beta^+$ HPFH¹⁰. In support of this hypothesis, a southern Italian with a phenotype similar to Greek HPFH has been found to carry a point mutation at -196 of the $\text{A}\gamma$ gene²⁷. Whether the -117 mutation described here is part of the same regulatory element as these, or a separate one, is unknown. All of these observations, however, point to the involvement of sequences 5' to the γ -globin genes in haemoglobin switching, perhaps by their interaction with a *trans*-acting repressor substance which is developmentally regulated.

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Living with penicillin

Jean Medawar

Howard Florey: Penicillin and After. By Trevor I. Williams.
Oxford University Press: 1984. Pp. 404. £17.50, \$25.

FEW scientists lead lives multifarious enough to support the structure of more than one serious biography within a few years of their death. Howard Florey died in 1968. Within 16 years the Royal Society's Biographical Memoir, Gwyn Macfarlane's fascinating biography (*Howard Florey: The Making of a Great Scientist*), an Australian life of Florey for general readers and, most recently, Trevor Williams's book have all been published — and no wonder to anyone who knew him.

Many readers of Macfarlane's book put it down with a feeling that they would recognize Florey if he walked into the room, that they had acquired a grasp of his career and stature. So why should there be any need for another? I started Trevor Williams's book with a "more of the same" feeling, but I lost it pretty soon, because the two books study different aspects of Florey's life and in different depth.

On inspecting the Florey archives at the Royal Society, Macfarlane realized that, for him, a full-scale biography was out of the question. So he wrote in detail about Florey's development as a scientist, up to the climax of the penicillin work in 1942, and condensed his subsequent career into an epilogue. Trevor Williams embarked upon *Howard Florey: Penicillin and After* to do justice to every facet of Florey's life and to complement Macfarlane's account, which he does, with thoroughness. His book makes it clear that Florey would be classed as a great man even if he had not rediscovered penicillin — by his peers, though not by the public. By 1944 when penicillin vials were rolling off the production line at Pfizer, faster than anyone could count, he had a quarter of a century still to go; in that time he travelled, in the train of penicillin, to America, Russia and Australia, laid the foundations of the Australian National University, was invited to assume and turned down the position of its director, was involved in the development of cephalosporin in his laboratory, became an extremely influential President of the Royal Society and Provost of Queen's College Oxford. He did all this without the support of either good health or the relaxation of domesticity.

Trevor Williams writes as a professional scientist. He worked in Florey's laboratory during the War, and was encouraged and helped in the writing of his book by Margaret Jennings, an important member of Florey's penicillin team, whom Florey married in 1967. A general reader may find that he is told more than he wants to know

about the chemical procedures that went into the early production of penicillin in the pathology laboratories at Oxford; but the story is powerful enough to sweep any reader forward over the details.

The book is crammed with absorbing stories which take up more than half of the pages. Fleming's first observation of penicillin as an antibiotic, Florey's hunch that Fleming's ten-year-old observation was worth following up, and Florey's indomitable pursuit of penicillin against dispiriting difficulties (in 1939 the Medical Research Council gave him only £25 for his proposed study of microbial antagonism). Of events that followed, the story that the Americans "stole" penicillin from the British needs to be retold. The accusations began around 1944; the complaint was that the British penicillin team had not patented their discovery "because in the medical field this was unethical". When the United States came into the war in 1941, the British freely handed over all they knew about the production and use of the drug, and the whole business was turned over to the Merck, Squibb and Pfizer companies. These companies produced quantities of penicillin by deep (as opposed to British surface) culture, and patented the process. So, after the War, British firms employing the deep technique had to pay royalties to the American firms.

John T. Connor, formerly the General Counsel of the United States Office of Scientific Research and Development, was asked to undertake a detailed enquiry into the matter. His 12-page report appeared in October 1952 and the summary of his conclusions, quoted on p. 311 of Williams's book, was uncompromising:

Only a misunderstanding of scientific research procedures, of patent law, of the facts and understandings surrounding the collaborative development of penicillin from an academic discovery to large scale commercial production, and of the parts played by the Americans and the British . . . could lead people of good will to say that America 'stole' penicillin from the British.

He concluded that the complicated story was not a simple one of theft, but was a "happy example of Anglo-American collaboration".

In addition to stories, the book provides a compelling study of the interaction between character and opportunity in science, and the influence of luck or chance on both. The theme of chance, in particular, runs thread-like through the pages.

If Fleming had not left an unincubated Petri dish seeded with staphylococci on his bench when he went on holiday, if weather conditions had not favoured the relative growth of both bacteria and the contaminating mould, if Fleming's mind had not been prepared to recognize what he saw on his return, penicillin might not have been discovered as an antibiotic for years. Again, if the techniques for extracting penicillin in 1929 had not been so complicated, Fleming *might* have gone on to do what Florey later succeeded in doing. Then, in 1935, if Sir Edward Mellanby had not arrived to turn the tide of opinion in the committee selecting the new Professor of Pathology at Oxford (his train was two hours late and the committee had almost decided in favour of another candidate) Florey would not have been in charge of splendid laboratories, attracting a splendid crew, ready to take on the study of penicillin.

In retrospect, it seems inevitable that Fleming and Florey should have reacted to events as they did. Fleming's mind was open to recognize what he saw in 1929, but his enthusiasm for developing its possibility was minimal. In later life he let it be known that only lack of resources had stood in his way, but by then adulation may have deceived him. He certainly seems to have inhaled deeply the flattery of the Fleming myth. By contrast Florey's enthusiasm and determination moved mountains and his dislike of publicity was largely the reason that his name is still not properly connected with penicillin. I think Florey would have liked Dr Williams's book, and I cannot give a better recommendation than that.

I can add only a small point and a small anecdote. Florey foresaw that the life-saving effect of antibiotics would massively increase the population of the world, and did what he could to draw attention to the danger. When I invited him to become



Florey (right) in cheerful mood on the eightieth birthday of his old friend Paul Fildes in 1962.

From *Howard Florey: Penicillin and After*.

President of the Family Planning Association he accepted, saying rather wryly (as I was told) "I can refuse her nothing" — because I had been able to help the first Lady Florey when she was ill; but everyone knew that if he had not approved, he would have refused. He also set up the Royal Society's Group on Population, jointly with the United States Academy of Sciences, and the group educated many of its august members.

As Trevor Williams describes, Florey was very good at welding the work of a research team. Male and female and younger and older members were all called



From Howard Florey, *Penicillin and After*.

The Nobel Laureate line up, 10 December 1945. Flanking Ernst Chain (centre) are Florey (on Chain's left) and Fleming.

Geheimerat, and given a chance to improve their education. I was once called in to help in an operation to obtain pure lymphocytes from the thoracic duct of a rabbit. Several large veins had to be dissected and tied off to make a small chamber from which the lymph could be collected. Professor R.D. Wright was doing the surgery when his scalpel slipped, flooding the field with scarlet. He looked up and drawled, as if to explain a sort of simian clumsiness, "In Orstralia ... they call me Panzee Wright" (not "Pansy", as recorded by Dr Williams; that nickname would have been quite inappropriate).

It is a pity that in spite of all that has been written about Fleming, Florey and penicillin, the true story has not yet trickled down to common knowledge. Recently I asked the headmaster of a very good preparatory school if he knew much about Lord Florey. He apologized for not knowing the name, because he was "not scientifically trained". Then I asked two boys, one 12, one 16, at different schools, what they knew about penicillin. "Oh yes", they both said, "it was discovered by Fleming"; "Alexander, not Ian" the younger one added. □

During 1937 Jean Medawar worked on a BSc thesis in the Dunn School of Pathology, Oxford, under the supervision of Florey. With P.B. Medawar she is author of Aristotle to Zoos: A Philosophical Dictionary of Biology, published last year.

Dispatched from the trenches

Steve Blinkhorn

Perspectives on Bias in Mental Testing.

Edited by Cecil R. Reynolds and Robert T. Brown.

Plenum: 1984. Pp. 608. \$60, £47.50.

PUBLIC policy and academic debate, in tackling the same issue, leave tangled skeins of consequence whose unravelling is best left to history. Here we have a case in point. No serious challenge now remains to the proposition that, with few and unimportant exceptions, mental tests are not biased against social and ethnic minority groups as a necessary consequence of their methods of construction. The evidence for this assertion is to be found in this collection of extended essays — and as much in what is lacking in the claims put forward by those contributors whose concern is primarily for the interests of the minority groups, as in the strength of the arguments adduced by the defenders of the tests. Yet, in all likelihood, courts will continue to pronounce inconsistently; educational policy and practice will continue to develop as though it had been demonstrated that a pernicious racial and class bias necessarily lies at the heart of the very methods which have shown themselves least susceptible to manipulation by the affluent white middle class.

Five years ago such a point of view would have seemed at least eccentric, at worst deliberately inflammatory. What has changed? The publication of Arthur Jensen's *Bias in Mental Testing* in 1980 was most certainly a turning point, not so much because of the quality of the work (even the most favourable reviews expressed reservations) as because it provided a focal point for debate. Jensen, who is also a contributor to this book, is not one to write ten words when a hundred will do, nor is he above lecturing his audience as though they were indolent sophomores. In consequence he presented a large, slow-moving target, and those of us who appreciated his enterprise whilst not always being totally convinced of his motives sat back and waited for the flak to start.

Flak there has been, but oh how lacking in aim and quality. Critics, here and elsewhere, have written as though Jensen had meretriciously selected and culled the research literature to support his point of view, but they have signally failed to document the extensive body of contradictory evidence which, one would suppose from the tenor of their arguments, must exist. There is sufficient eccentricity in Jensen's view of the technicalities of psychometrics to reveal chinks in his statistical armour, but the critics aim four-square at solid steel plate. Indeed, from the example of one interpretation of statistics

presented here one must allow that Jensen has not under-estimated his critical audience. To read a scientist (even a social scientist) interpreting the difference between a statistically significant result in one group and a non-significant result in another as of itself necessarily significant is not edifying. The suggestion that thereafter *post hoc* comparisons should be made as if they had been planned and the results interpreted accordingly does nothing to repair the damage.

Furthermore, labouring quietly but effectively in the unsung fields of industrial psychology, Messrs Hunter and Schmidt have in the book performed an invaluable function. Put quite simply, they have shown that such differences as exist between groups in the regression of criterion performance on test scores are attributable overwhelmingly to intercept differences. Insofar as there is a difference, the problem is not that tests under-predict the performance of blacks (and in general the minority group in question is North American blacks), but that they over-predict it. In other words, in predictive terms, there is an argument for saying that tests are biased against whites. This book is worth its cover price for the cool exposition of this line of work, and for the concomitant examination of possible ethical positions with regard to the application of psychometric tests for practical purposes.

Sandwiched between Hunter and Schmidt's initial chapter and Jensen's closing commentary on the contents is a pot-pourri of contributions of varying degrees of enterprise, vigour, self-consciousness, competence, irrelevance, tedium, teasing possibility and sheer length. Most are attractive in some degree, and all breathe honest conviction. Unfortunately breathing conviction is not always the same as carrying conviction.

For instance, in an elegant and almost captivating experiment with rats, Harrington demonstrates that what the textbooks say is involved in developing a psychometric test will, when applied to maze-learning in rats, most likely give rise to a test which favours the strain of rat on which it has been developed. In practice, results with human subjects are not congruent with those from the rat model. (The question as to whether what test constructors actually do is fairly represented by what the textbooks say they do is another matter altogether.) Most notably there is now an accumulation of evidence that ethnic Chinese and Japanese have higher mean scores on conventional tests developed by Europeans and Americans than do Europeans and Americans themselves. The quality of these data is somewhat variable but, taken together with other group mean data, it is clear that being white and middle-class does not guarantee a place at the top of the IQ heap.

Six hundred or so pages on one is left with a feeling of unsatisfied curiosity, almost as if one had been present at a debate

in a group whose interests, social conventions and special vocabulary one did not share. What actually is going on? Not so much a discussion concerning bias in mental testing, more an attempt to work through some of the difficulties arising from the custom of classifying both oneself and others as belonging to a specific racial group, and most particularly the consequences in the United States. Sex and social class differences don't even merit entries in the index. Of course it is clear why racial differences, particularly when group membership can be imputed on cursory inspection of the face, have dominated the argument. But when arguments run dry and the evidence on either side gets thin, prejudices surface in the description of inconclusive data by all sides in the debate. It is difficult to shake off the impression that with few exceptions pro- and anti-test contributors alike seek support from research for positions they would hold to regardless of the evidence. The fact that at present the weight of evidence is firmly on the side of the hypothesis of no bias does not dispose of a sense of unease about why these authors take such an interest in the question of racial differences in the first

place. One scarcely wants to see ethnic origin as the principal defining characteristic of one's fellows.

Invariably linked with the test bias issue, although for no good logical reason, is the question of the heritability of IQ. It is to be regretted that a slippery slope of unreason leads from the affirmation of no test bias to the assumption that IQ differences across races are fixed genetically and not susceptible to modification, and thence to the conclusion that blacks are an inferior breed. It is surely incumbent on those whose reputations rest on their publications in this field to signpost very carefully and clearly the limits of the arguments they put forward. Equally, those who continue to assert the presence of bias should clearly state whether it is only ethnic groups which must, *a priori*, have equal mean scores on tests, or whether all group memberships are necessarily random with respect to IQ.

One can only welcome the appearance of this book: how many will have the stamina to read it all is another matter. □

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Standard physics

C.J.S. Clarke

Supermanifolds. By Bryce DeWitt.
Cambridge University Press: 1984.
Pp.316. £35, \$59.50.

THE main difficulty in reviewing this book was fending off colleagues and students trying to get their hands on it. For, although supermanifolds have played a large part in theoretical physics over the past few years (and are predominant in modern theories of quantum gravity), this is the first textbook to be devoted to the subject. Having been circulated in part as dog-eared photocopies for some time, the finished version has been eagerly awaited.

The book begins inauspiciously, however. There are now five essentially different definitions of supermanifolds on the market (those of Kostant, Rogers, Jadczyk-Pilch, Batchelor and DeWitt). A few conjectures and theorems link them, but they differ in the choice of the definition of "differentiable" when applied to functions taking values in a Super-Euclidean space made up of both commuting and anticommuting coordinates. Here, although some of the other contenders are given a passing reference, there is nothing to suggest to the reader initially that there could be any alternative to DeWitt's own theory. Differentiability is swept away on p. 3 where the author first asserts that his basic (infinite dimensional) vector space has neither norm nor topology, and then proceeds to differentiate functions on it!

But this problem in no way detracts from the excellence of the subsequent exposition of the geometry of supermanifolds. All the properties with which workers in general relativity are familiar, including connections, geodesics and curvature, are dealt with for supermanifolds in a strongly geometric spirit, using a very efficient notation. Super-Lie groups are given a most thorough treatment, including specification of the analogues of the classical Lie groups. The book ends with a discussion of physical applications to Bose-Fermi-interchanging symmetries. Here some might harbour mathematical doubts as to the propriety of some of the manipulations of path-integrals, but in comparison with the standards of mathematical validity prevailing in much of theoretical physics today DeWitt's treatment looks positively rigorous.

In the scope of its geometric development, DeWitt's analysis goes well beyond that of the other theories mentioned above. And at the level of basic structure there are several interesting additions not found in the mathematical literature, such as the stress on the use of complex super-vector spaces having a conjugate-linear automorphism, and the systematic use of super-Hilbert space for quantum mechanics.

Each chapter contains a set of exercises, making it possible to use the work as a self-contained graduate text. *Supermanifolds* is destined to become the standard work for all serious study of super-symmetric theories of physics. □

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Something missing

George Efstathiou

The Hidden Universe.

By Michael Disney.

Dent/Macmillan, New York: 1984.
Pp. 216. £10.95, \$16.95.

It was about time that a popular book appeared on the "missing-mass" problem. There is now considerable evidence that more than 90% of the mass of the Universe is invisible, but nobody knows what this dark stuff is made of. Is it dead stars, low-mass "stars" which never ignited, hot gas, black holes or perhaps elementary particles such as neutrinos?

Michael Disney explores all of these possibilities, summarizing the various observational limits and theoretical arguments which have narrowed the range of acceptable candidates. But in my view he fails to convey the great sense of excitement and the rapid progress that have characterized recent years. For example, current observations imply that the average density in the Universe is about a tenth of the critical density predicted by Einstein's General Theory of Relativity. Isn't it possible that undetected dark matter can make up the difference? This is certainly Disney's view, but it is given additional respectability by new theories of the early Universe — the so-called inflationary models. It is a shame that these are only briefly mentioned in the last few pages of the book.

Moreover, the discussion of particle theories and their impact on modern cosmology is far too scant. Disney gives the impression that nearly all the proposed candidates suffer from some weakness and that we should consider more radical alternatives, such as abandoning Newtonian gravity. However, some of his theoretical arguments are either weak or wrong. For example, Disney argues that massive neutrinos would cluster strongly around galaxies, but he ignores phase-space restrictions and the fact that neutrino fluctuations are damped on galactic scales. Further, many exotic forms of dark matter, such as gravitinos, photinos and axions, are not even mentioned.

Of course it is all too easy to criticize. The author has clearly spent a great deal of time in carefully constructing analogies to explain complex points to the layman; the sections on stellar evolution are particularly clear. Disney presents a good picture of modern observational cosmology — the difficulties, the uncertainties and the controversies — and if some of the more glaring errors and omissions were corrected this would be an excellent book. □

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Naming names among the yeasts

James A. Barnett

The Yeasts: A Taxonomic Study, 3rd Edn.

Edited by N.J.W. Kreger-van Rij.
Elsevier Science: 1984. Pp.1,082. \$173,
Dfl.450.

THE 16 contributors to this book are among those who know most about yeast taxonomy today. So they are well qualified for the primary aim of the work (p. vi), namely, to present and discuss the classification of this immensely important group of fungi.

Praise should be given to the excellent descriptions with drawings of the sexual states of the teliospore-forming yeasts *Sporidiobolus*, *Rhodospiridium* and *Leucosporidium*. The descriptions are especially valuable as these states are difficult to observe.

There are some clear and helpful explanations of why certain yeast names have been changed or alternative names preferred. For example (p.315), Kurtzman retains *Pichia burtonii* and has not put this yeast in either *Hyphopichia* or *Saccharomycopsis*, as has been proposed, (i) because the simple closure line of the hyphal septa is typical of other mycelial *Pichia* species and (ii) with the removal of *Saccharomycopsis lipolytica* to the genus *Yarrowia*, all remaining *Saccharomycopsis* species have septa with plasmodesmata. It is, incidentally, very odd indeed that *Yarrowia*, although published as long ago as 1980, is not discussed in the book.

On the other hand, there are also many instances in which the reader is left with too much to work out for himself. For example, it would be helpful to be told why *Arthroascus javanensis* (pp.114–116), formerly belonging to the genus *Endomycopsis*, should be singled out and given its own separate genus. Unprompted, the reader must turn to a table on pp.26–27 to find the explanation. Another example is provided by *Endomycopsis* itself which, although accepted in the second edition, is now considered an invalid genus (p.413); but no explanation is offered.

More irresponsible than failures to explain changes in classification are the continual alterations of nomenclature by yeast taxonomists. This practice is analogous to that of the frequent alteration of street names to accord with some political fashion: all perfectly rational, but damnable and annoying and confusing. Here, for example, Kurtzman (Chapter III) describes 30 species of *Hansenula*, but in a paper also published in 1984 he had the temerity to abolish the whole genus! Such practices make it difficult to persuade experimental microbiologists that taxonomists should be taken seriously.

The secondary aim of the book (p. vi) is to aid identification, and the methods chapter is packed with helpful instructions for isolating, maintaining and identifying yeasts. However, the standard description of each species is now much less informative than in the previous edition; for example, *Schizosaccharomyces pombe* was then described by results of 51 tests, but now by only 29. Less information leads to greater difficulty in the process of identifying and, moreover, if there are now just enough tests for distinguishing between existing species, it cannot be known which of the discarded tests will prove useful for discriminating newly discovered yeasts. Furthermore, the number of tests used in the standard descriptions varies markedly from one genus to another, so that it is difficult to know which tests to do, unless the genus is known

first. Generally, it would have been helpful to have been told which strains formed the basis for each standard description.

There are no photographs in the book, only line drawings; while these can be better than photographs, why are only about 60% of the species illustrated? And although in this edition there are more identification keys than formerly, most of them are difficult to use because of bad layout and excessive abbreviation of the test names.

This edition of *The Yeasts* is more an academic work than a practical manual for identification. It is, however, authoritative and will be essential reading for yeast taxonomists. □

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From the bottom

Peter D. Moore

Lake Sediments and Environmental History: Studies in Palaeolimnology and Palaeoecology in Honour of Winifred Tutin.

Edited by Elizabeth Y. Haworth and John W.G. Lund.

Leicester University Press/University of Minnesota Press: 1984. Pp.411. £39, \$55.

MULTI-author *Festschrift* volumes, written in honour of esteemed scientists, are all too often uncoordinated collections of essays covering a disparate range of subjects. The editors of this assemblage of palaeoecological articles have been able deftly to avoid the problem. Professor Winifred Tutin's interests, and hence the subjects covered in the book, are centred upon lakes and the stories which can be unravelled about the past from their stratified sediments. With this as a central and well-defined theme, there is ample scope to illustrate the wide range of evidence which can be used in studies of environmental history and the subject is tackled both by means of review papers and by case-studies of selected sites.

Pennington and palynology are inseparable in most people's minds, so it is natural that pollen analytical articles should form an important component of the book. These contributions reflect the recent changes in emphasis within the discipline, from the accumulation of data on pollen stratigraphy to an understanding of the processes involved in pollen movement and deposition. So we have essays on pollen recruitment to lakes by Bonny and Allen, and on variation in pollen deposition rates in different parts of lake basins by Davis, Moeller and Ford. Turner shows the value of principal-components analysis in her comparison of peat profiles on Cross Fell, which was conducted in order to determine

the altitude of past tree lines. She comes to the conclusion — contrary to expectation — that this Pennine peak of 893m carried an open birch woodland during the Flandrian climatic optimum. An analogous study by Watts of the Burren in western Ireland also shows that this area was forested prior to the advent of prehistoric agriculture.

In her contribution, Hilary Birks demonstrates how small lakes — a somewhat neglected resource for palaeoecologists — have some advantages over larger ones in providing macrofossil evidence of local vegetation changes. She describes the late glacial stratigraphy of such a site in the far north-west of Scotland. Soil profiles provide an even more local picture of environmental change, as shown in the chapter by Andersen which describes his studies in Denmark.

The physical and chemical properties of lake sediments also receive an airing. Of particular interest is Cranwell's discussion of the organic geochemistry of lake sediments, concentrating on pentacyclic triterpenes; by means of these compounds he claims to be able to distinguish an allochthonous input of peat from that of forest humus. Finally, for the theoretician, there is a contribution by Deevey on stability in lake ecosystems. Deevey is both modest and generous in referring to theoretical ecology as being "primitive rather than sick", and he makes out a strong case for the use of lacustrine systems as a basis for successional and stability studies.

The essential unity of this volume will give it a clear market among research workers in limnology and palaeoecology. As a series of examples of current advances in techniques in these disciplines it is also a book to which undergraduates could profitably be directed. □

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The importance of photographic gelatin

from Yves Lestra

The demands of modern photography have led to the evolution of extremely sensitive photographic emulsions. The vital role of gelatin in the emulsion is easily overlooked.

IN the emulsion of a photographic film, silver chloride and bromide darken and decompose into their constituent parts when exposed to actinic light. This darkening is a very slow process unless it is accelerated chemically by use of a reducing solution or 'developer', which liberates silver from the exposed grains without affecting unexposed grains. This process furnishes most of the energy necessary to obtain an image. It is worth considering how an emulsion is produced, because the properties of the emulsion, almost always containing gelatin as the major colloid, are crucial in determining the quality of the image obtained.

Emulsion manufacture

The initial step in the manufacturing process involves the precipitation of silver chloride or bromide crystals from solutions of alkaline bromides and silver nitrate. The reaction takes place in a colloidal medium in order to keep the insoluble crystals of the silver salts in suspension.

The emulsion then undergoes a period of physical ripening or ageing during which the crystals grow larger. Any soluble salts remaining in solution are then removed by washing. For many years this involved gelling of the colloidal suspension and dialysis of the soluble salts by washing the gel in cold water. Today, most manufacturers rely on flocculation of the colloid by salts or organic reagents, including the modified gelatins, which flocculate in acid conditions.

Next comes a second, or chemical ripening. At this stage certain critical additions are made to encourage the formation of sensitivity centres on the crystals of the silver salts.

Finally, various additives are used to stop the ripening process, to encourage the coating of the emulsion and to harden the emulsion and make it insoluble to the developer. The emulsion is then coated into its support and cooled. It is coated with a protective layer of gelatin which is photographically inert.

Role of gelatin

Gelatin is generally the colloid of choice for the manufacture of emulsions. During precipitation and physical ripening it keeps the silver salts in suspension and is strongly absorbed at the surface of the silver halide crystals, retarding their growth. The ability of gelatin to form a gel allows the elimination of unwanted soluble salts by dialysis, and its high viscosity facilitates the coating of the emulsion onto the support.

After coating, the gel formed keeps the silver salt crystals on the support during drying and development, and when the emulsion is exposed to light, the gelatin prevents the inverse reaction of the photolysis by fixing the liberated halogen. Gelatin also prevents fading of the latent image resulting from the photolysis.

During development, the swelling of the gelatin, caused by the action of the added reagents, allows the reagents to diffuse through the emulsion layer. The property of the gelatin to be tanned by various substances allows rapid development at high temperatures.

Since gelatin is extracted from the collagen of bone or skin, most preparations inevitably contain impurities. Some are detrimental to the photographic process and must be removed (dust, heavy metals, grease and 'fogging agents'). Albumens, polypeptides containing cystine and nucleic acids act as 'restrainers', blocking the formation of sensitivity sites or inhibiting the development of silver halide grains. Some impurities, however, have useful properties acting as 'sensitizers' and facilitating the production of silver or silver sulphide centres on the halide grains.

Photographic gelatins can be classified into four groups: Inert gelatin, without sensitizers or restrainers; active gelatin, rich in sensitizer and low in restrainer; retarded gelatin, low in sensitizer and rich in restrainer; and retarded active gelatin, rich in both sensitizer and restrainer.

The task of the photographic emulsion manufacturer is to find the best gelatin for a specific application. The gelatin manufacturer must produce the gelatin to an exact specification and ensure that quality is maintained from batch to batch.

Gelatin production

The conditions under which gelatin extraction occurs determine the properties of the end product. In particular, the liming process has to be closely controlled. Chemical changes occurring at this stage include the hydrolysis of amide groups to carboxylic groups (destroying the triple helix of collagen), the elimination of many impurities (mucopolysaccharides, tyrosine, sulphur), the transformation of the sulphur in cystine to thiosulphate and the degradation of ribonucleic acid to nucleotides.

After liming, the raw material is washed with water to eliminate as much lime as possible, and then 'delimed' in a bath of dilute acid. Gelatin is then extracted in hot

water, with only the first extraction pure enough for the exacting requirements of photography.

Modified gelatins

It is possible to chemically modify gelatins in order to provoke flocculation simply by lowering the pH. This type of gelatin is increasingly used for flocculation of emulsions, replacing sodium or ammonium sulphate or washing by dialysis.

Auxiliary gelatins

Photographic materials do not consist of a single light sensitive layer. Other layers—auxiliaries—are coated onto the support before, or at the same time as, the emulsion layer. The support itself is usually synthetic and its surface has little affinity for the hydrophilic emulsion. An intermediate layer or substratum is required, containing the necessary ingredients to establish a bond between the emulsion and support.

Colour films or papers contain several emulsion layers, each sensitive to a different radiation band. The layers must be separated by intermediate layers to prevent diffusion from one colour layer to another.

The surface of the emulsion layer must be protected mechanically from external contact. This is the role of the supercoat. Like all auxiliary layers, it must have no photographic influence on the emulsion. They are commonly inert gelatines.

In order to compensate for the tension created on one side of the support by all the layers, a backing layer is coated on the other side of the support. This is often coloured in order to avoid the halo created by the light ray as it reflects on the film. Some colour films can contain as many as a dozen layers of chemicals. The sophisticated coating machines in use today allow the simultaneous coating of most of the layers at speeds of 100 to 150 metres per minute. Each layer is only some 10 μm thick, so it is easy to appreciate the importance of the physical qualities required of the gelatin.

To supply the customer with a photographic gelatin with precisely the properties required, the manufacturer must be able to control the production process very accurately, and quality control must be stringent. In addition, it is useful for the customer to appreciate the problems of the manufacturer when specifying requirements for an emulsion. □

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Photography and imagery

This week's product review covers some of the latest advances in photomicrography and processing of micrograph images.

● The rapid developments in imaging technology have led to greater demands on the recording materials available. To meet these demands, Kodak has introduced 'T-Grain' emulsions, incorporating carefully oriented flat silver halide grains that allow much faster emulsions, without the usual trade-off in graininess. This is incorporated in Kodak's latest colour negative films, and will soon appear in X-ray films also. Kodak's range of materials for scientific use includes papers for recording oscilloscope traces and plates for electron microscopy, photomicrography, holography, spectroscopy and astronomy. High resolution plates, for example, are capable of recording over 2,000 lines per mm. Kodak Technical Pan film 2415 ('Ester-AH' Base) is particularly useful, allowing enlargements of $\times 25$ and more with little perceptible grain. Also from Kodak, the Sp2000 motion analysis system achieves video recording of high speed events at between 60 and 2,000 frames per second. The frame may also be divided into up to 6 horizontal segments, providing 12,000 frames per second.

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● Bausch & Lomb has published two new brochures that describe their video-based image analysers. Common applications of image analysis are: bacterial colony counts, airborne contaminant analysis, fibre measurement in the textile industry, aerial and satellite photograph analysis and structural integrity analysis of building materials.

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● Dapple System, Inc. has published an image analysis brochure describing the Image-Plus+ system for automatic measurement of features in video images. The various methods for producing the image signal, from an optical microscope, SEM, or TEM, are detailed and stereological analysis techniques discussed. The descriptions include the method of acquisition and storage in the computer memory of the grey-scale image and the image array processing functions available to enhance this image before measurement. Data processing and statistical analysis routines required for quantitative stereology are described and illustrated by example.

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The SZH in a configuration for photomicrography.

● The building-block design of the new Olympus SZH zoom stereo microscope system makes it especially suited for photomicrography. As well as the PM Series photomicrographic system, Olympus OM Series SLR cameras can be fitted. The binocular viewing tube, inclined at 45° , is designed to permit easy focusing for photomicrography, and the distortion-free objective lenses, in magnifications of 0.5, 0.75, 1, 1.5 and 2, provide clear images without the need for auxiliary lenses. Eyepieces are available with four different magnifications, 10, 15, 20 and $30\times$.

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● Photonics Microscopy, Inc. of Illinois has introduced the Model C1966, a high-speed video image processing system designed for real time applications. The C1966 features analog video enhancement and digital image processing through frame memories that permit subtraction, averaging, filtering and other image processing functions. These techniques make it possible to view microscopic structures and events that cannot be seen in the optical image. The C1966 can be used with video cameras and X-ray through infrared wavelength sources can be accommodated through the selection of specific cameras. Three frame memories of 640 by 438 lines permit extensive image manipulation in real time on the video monitor, while the image may also be recorded permanently on videotape or videomicrograph. The C1966 will interface with most popular computers.

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A human embryo after 12 weeks of differentiation, photographed by Dr R. Jonas. The instrument used was the Wild Heerbrugg M 400 Photomakroskop. The M 400 has been specially developed for 'macroscopy', that is, for the magnification range of 1:1 to 20:1, where the close-up range of an ordinary camera is no longer adequate, but where the visual field of the

microscope becomes too small. The photographic range of the M 400 is in fact from 1:1 to 60:1, and there is a macrozoom objective (1:5) to provide 'stepless' changes in magnification. Further features of the P 400 include binocular observation, working distances of 42-188 mm and an automatic system to determine exposure.

Circle No. 103 for further details.

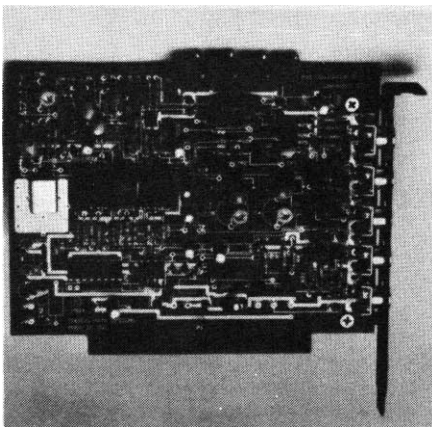
These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● The 3090 pulsed light fibre-optic sensor from Oriel Scientific is designed to make a measurement of fluorescence, absorption, reflection or scattered light using fibre-optic probes called 'optrodes', and will make the measurement in moderate ambient light. A full range of glass, silica, and liquid light guides allows measurements to be made in radioactive areas, fermentation systems, high voltage or explosive areas, biological hazard areas and marine locations. The analyser has its own control logic and will interface to a micro-computer such as the PET or Apple II.

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● Signals passed down optical fibres will not reach their destination if there is a break in the cable. Now that optical fibres are widely used in communications equipment, accurate methods of precisely locating breaks are extremely important. One such technique is optical time domain reflectometry (OTDR) which is capable of providing accurate information on faults in cables several metres in length. EG&G Instruments have recently announced their Model 4400 Boxcar, a system that incorporates signal processor, boxcar averager and gated integrator in a single unit, for use in OTDR experiments. OTDR relies on measuring the backscatter of a light beam that is passed down a fibre optic cable. Since the signal power and backscatter levels are very low, sophisticated timing electronics and signal processors are needed.

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The Colorverter from Chorus

● New from Chorus Data Systems, Inc., the Colorverter is a low-cost converter which changes NTSC composite colour signals into analog RGB. Colorverter may be used in conjunction with the Chorus PC-EYE Video Capture System to digitize colour signals from colour video cameras, VCRs, and laser disks. The user has control of the colour intensity, tint (hue), brightness, and contrast. EIA RS-170A sync is available on all signals and there is a separate monochrome output for black-and-white applications. Applications include slide generation, inspection (where colour distinction is important) and medical/scientific classification. Systems and software support for many of these applications are available.

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● Several different imaging techniques are incorporated in the Kevex Microanalyst 8000 energy dispersive X-ray analyser for electron microscopists. Imagex is a sophisticated software package that allows the characteristic X rays and other electron column signals to be digitized, stored and manipulated. Images are divided into a $1,024 \times 1,024$ pixel grid and each pixel can be stored as one of up to 64,000 levels. The Kevex Microanalyst 8000 is also able to display colour images. Different colours can be designated to different elements, thereby allowing accurate and easy to interpret displays, photographs or printer outputs to be produced.

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● The Model 850 is a new image analysis system for biomedical research from Spatial Data Systems. It is a complete turn-key system for statistics database management and image capture, enhancement, segmentation, analysis, storage and retrieval. The hardware is configured to allow the operator to input images from film or microscope slides, to enhance them and gather statistics, and then to store on disk, display or print the results. The new system includes a camera, light table, alphanumeric terminal, colour monitor, joystick interface, printer, LSI II CPU, Winchester technology disk, eight-inch floppy disk, an EyeCom II real-time image processor, desk workstation and complete software. The system is available in a range of configurations.

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